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(54) Title: COMPOSITIONS FOR TREATING INFLAMMATORY RESPONSE

(57) Abstract

Compounds and methods are provided to treat inflammatory conditions with certain A2A adenosine receptor antagonists.

COMPOSITIONS FOR TREATING INFLAMMATORY RESPONSE

Field of the Invention

5   The present invention relates to methods and compositions for preventing tissue injury, i.e., due to inflammatory activity.

Background of the Invention

The present invention was made with the assistance of U.S. Government funding (NIH Grant ROL HL37942). The U.S. Government has certain rights in

10   this invention.

The inflammatory response serves the purpose of eliminating harmful agents from the body. There is a wide range of pathogenic insults that can initiate an inflammatory response including infection, allergens, autoimmune stimuli, immune response to transplanted tissue, noxious chemicals, and toxins,

15   ischemia/reperfusion, hypoxia, mechanical and thermal trauma. Inflammation normally is a very localized action which serves in expulsion, attenuation by dilution, and isolation of the damaging agent and injured tissue. The body's response becomes an agent of disease when it results in inappropriate injury to host tissues in the process of eliminating the targeted agent, or responding to a

20   traumatic insult.

As examples, inflammation is a component of pathogenesis in several vascular diseases or injuries. Examples include: ischemia/reperfusion injury (N. G. Frangogiannis et al., in Myocardial Ischemia: Mechanisms, Reperfusion, Protection, M. Karmazyn, ed., Birkhuser Verlag (1996) at 236-284; H. S.

25   Sharma et al., Med. of Inflamm., 6, 175 (1987)), atherosclerosis (R. Ross, Nature, 362, 801 (1993)), inflammatory aortic aneurysms (N. Girardi et al., Ann. Thor. Surg., 64, 251 (1997); D. I. Walker et al., Brit. J. Surg., 59, 609 (1972); R. L. Pennell et al., J. Vasc. Surg., 2, 859 (1985)), and restenosis following balloon angioplasty (see, R. Ross cited above). The cells involved with inflammation

30   include leukocytes (i.e., the immune system cells – neutrophils, eosinophils,

1

lymphocytes, monocytes, basophils, macrophages, dendritic cells, and mast cells), the vascular endothelium, vascular smooth muscle cells, fibroblasts, and myocytes.

The release of inflammatory cytokines such as tumor necrosis factor-alpha (TNFα) by leukocytes is a means by which the immune system combats pathogenic invasions, including infections. TNFα stimulates the expression and activation of adherence factors on leukocytes and endothelial cells, primes neutrophils for an enhanced inflammatory response to secondary stimuli and enhances adherent neutrophil oxidative activity. See, Sharma et al., cited above. In addition, macrophages/dendritic cells act as accessory cells processing antigen for presentation to lymphocytes. The lymphocytes, in turn, become stimulated to act as pro-inflammatory cytotoxic cells.

Generally, cytokines stimulate neutrophils to enhance oxidative (e.g., superoxide and secondary products) and nonoxidative (e.g., myeloperoxidase and other enzymes) inflammatory activity. Inappropriate and over-release of cytokines can produce counterproductive exaggerated pathogenic effects through the release of tissue-damaging oxidative and nonoxidative products (K. G. Tracey et al., J. Exp. Med., 167, 1211 (1988); and D. N. Männel et al., Rev. Infect. Dis., 9 (suppl. 5), S602-S606 (1987)). For example, TNFα can induce neutrophils to adhere to the blood vessel wall and then to migrate through the vessel to the site of injury and release their oxidative and non-oxidative inflammatory products.

Although monocytes collect slowly at inflammatory foci, given favorable conditions, the monocytes develop into long-term resident accessory cells and macrophages. Upon stimulation with an inflammation trigger, monocytes/macrophages also produce and secrete an array of cytokines (including TNFα), complement, lipids, reactive oxygen species, proteases and growth factors that remodel tissue and regulate surrounding tissue functions.

For example, inflammatory cytokines have been shown to be pathogenic in: arthritis (C. A. Dinarello, Semin. Immunol., 4, 133 (1992)); ischemia (A. Seekamp et al., Agents-Actions-Supp., 41, 137 (1993)); septic shock (D. N. Männel et al., Rev. Infect. Dis., 9 (suppl. 5), S602-S606 (1987)); asthma (N. M. Cembrzynska et al., Am. Rev. Respir. Dis., 147, 291 (1993)); organ transplant

rejection (D. K. Imagawa et al., Transplantation, 51, 57 (1991); multiple
sclerosis (H. P. Hartung, Ann. Neurol., 33, 591 (1993)); AIDS (T. Matsuyama et
al., AIDS, 5, 1405 (1991)); and in alkali-burned eyes (F. Miyamoto et al.,
Opthalmic Res., 30, 168 (1997)). In addition, superoxide formation in
5   leukocytes has been implicated in promoting replication of the human
immunodeficiency virus (HIV) (S. Legrand-Poels et al., AIDS Res. Hum.
Retroviruses, 6, 1389 (1990)).

It is well known that adenosine and some analogs of adenosine that
nonselectively activate adenosine receptor subtypes decrease neutrophil
10   production of inflammatory oxidative products (B. N. Cronstein et al., Ann. N.Y.
Acad. Sci., 451, 291 (1985); P. A. Roberts et al., Biochem. J., 227, 669 (1985);
D. J. Schrier et al., J. Immunol., 137, 3284 (1986); B. N. Cronstein et al.,
Clinical Immunol. and Immunopath., 42, 76 (1987); M. A. Iannone et al., in
Topics and Perspective in Adenosine Research, E. Gerlach et al., eds., Springer-
15   Verlag, Berlin, p. 286 (1987); S. T. McGarrity et al., J. Leukocyte Biol., 44,
411421 (1988); J. De La Harpe et al., J. Immunol., 143, 596 (1989); S. T.
McGarrity et al., J. Immunol., 142, 1986 (1989); and C. P. Nielson et al., Br. J.
Pharmacol., 97, 882 (1989)). For example, adenosine has been shown to inhibit
superoxide release from neutrophils stimulated by chemoattractants such as the
20   synthetic mimic of bacterial peptides, f-met-leu-phe (fMLP), and the
complement component C_5a (B. N. Cronstein et al., J. Immunol., 135, 1366
(1985)). Adenosine can decrease the greatly enhanced oxidative burst of PMN
(neutrophil) first primed with TNF-α and then stimulated by a second stimulus
such as f-met-leu-phe (G. W. Sullivan et al., Clin. Res., 41, 172A (1993)).
25   Additionally, it has been reported that adenosine can decrease the rate of HIV
replication in a T-cell line (S. Sipka et al., Acta. Biochim. Biopys. Hung., 23, 75
(1988)). However, there is no evidence that in vivo adenosine has anti-
inflammatory activity (G. S. Firestein et al., Clin. Res., 41, 170A (1993); and B.
N. Cronstein et al., Clin. Res., 41, 244A (1993)).
30   It has been suggested that there is more than one subtype of adenosine
receptor on neutrophils that can have opposite effects on superoxide release (B.
N. Cronstein et al., J. Clin. Invest., 85, 1150 (1990)). The existence of A_{2A}

3

receptor on neutrophils was originally demonstrated by Van Calker et al. (D. Van Calker et al., Eur. J. Pharmacology, 206, 285 (1991)).

There has been progressive development of compounds that are more and more potent and/or selective as agonists of A_{2A} adenosine receptors (AR) based on radioligand binding assays and physiological responses. Initially, compounds with little or no selectivity for A_{2A} receptors were developed, such as adenosine itself or 5'-carboxamides of adenosine, such as 5'-N-ethylcarboxamidoadenosine (NECA) (B. N. Cronstein et al., J. Immunol., 135, 1366 (1985)). Later, it was shown that addition of 2-alkylamino substituents increased potency and selectivity, e.g., CV1808 and CGS21680 (M. F. Jarvis et al., J. Pharmacol. Exp. Ther., 251, 888 (1989)). 2-Alkoxy-substituted adenosine derivatives such as WRC-0090 are even more potent and selective as agonists at the coronary artery A_{2A} receptor (M. Ueeda et al., J. Med. Chem., 34, 1334 (1991)). The 2-alklylhydrazino adenosine derivatives, e.g., SHA 211 (also called WRC-0474) have also been evaluated as agonists at the coronary artery A_{2A} receptor (K. Niiya et al., J. Med. Chem., 35, 4557 (1992)).

There is one report of the combination of relatively nonspecific adenosine analogs, R-phenylisopropyladenosine (R-PIA) and 2-chloroadenosine (Cl-Ado) with a phosphodiesterase (PDE) inhibitor resulting in a lowering of neutrophil oxidative activity (M. A. Iannone et al., Topics and Perspectives in Adenosine Research, E. Garlach et al., eds., Springer-Verlag, Berlin, pp. 286-298 (1987)). However, R-PIA and Cl-Ado analogs are actually more potent activators of A_1 adenosine receptors than of A_{2A} adenosine receptors and, thus, are likely to cause side effects due to activation of A_1 receptors on cardiac muscle and other tissues causing effects such as "heart block."

4

R. A. Olsson et al. (U.S. Pat. No. 5,278,150) disclose selective adenosine A_2 receptor agonists of the formula:

$$R_1R_2C=NNH \text{—[purine ring with } NH_2, N, N, N, N, Rib]$$

5
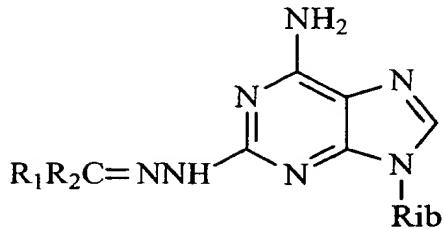
wherein Rib is ribosyl, R_1 can be H and R_2 can be cycloalkyl. The compounds are disclosed to be useful for treating hypertension, atherosclerosis and as

10   vasodilators.

Olsson et al. (U.S. Pat. No. 5,140,015) disclose certain adenosine A_2 receptor agonists of formula:

$$R_1\text{—O}, \; R_2\text{—B—C(X)—[ribose ring with } NH_2 \text{ purine, } O, OH, OH]$$
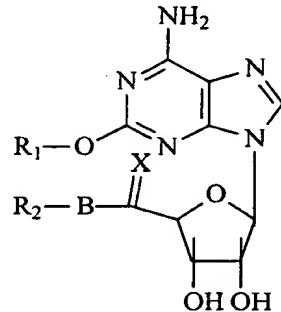
wherein $C(X)BR_2$ can be CH_2OH and R_1 can be alkyl- or alkoxyalkyl. The compounds are disclosed to be useful as vasodilators or an antihypertensives.

15   Linden et al. (U.S. Pat. No. 5,877,180) is based on the discovery that certain inflammatory diseases, such as arthritis and asthma, may be effectively treated by the administration of compounds which are selective agonists of A_{2A} adenosine receptors, preferably in combination with a Type IV phosphodiesterase inhibitor. An embodiment of the Linden et al. invention

20   provides a method for treating inflammatory diseases by administering an effective amount of an A_{2A} adenosine receptor of the following formula:
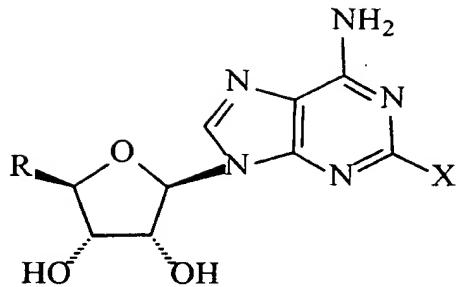
wherein R and X are as described in the patent.

In a preferred embodiment, the Linden et al. invention involves the administration of a Type IV phosphodiesterase (PDE) inhibitor in combination with the A_{2A} adenosine receptor agonist. The Type IV phosphodiesterase (PDE)

5   inhibitor includes racemic and optically active 4-(polyalkoxyphenyl)-2-pyrrolidones of the following formula:
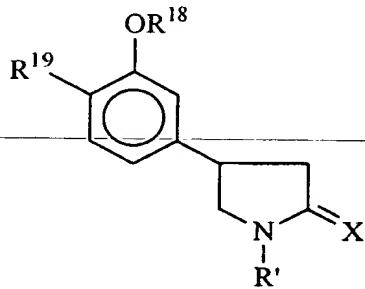
wherein R', R^{18}, R^{19} and X are as disclosed and described in U.S. Pat. No. 4,193,926. Rolipram is an example of a suitable Type IV PDE inhibitor included within the above formula.

10   G. Cristalli (U.S. Pat. No. 5,593,975) discloses 2-arylethynyl, 2-cycloalkylethynyl or 2-hydroxyalkylethynyl derivatives, wherein the riboside residue is substituted by carboxy amino, or substituted carboxy amino ($R_3HNC(O)$-). 2-Alkynylpurine derivatives have been disclosed in Miyasaka et al. (U.S. Pat. No. 4,956,345), wherein the 2-alkynyl group is substituted with

15   (C_3-C_{16})alkyl. The '975 compounds are disclosed to be vasodilators and to inhibit platelet aggregation, and thus to be useful as anti-ischemic, anti-atherosclerosis and anti-hypertensive agents.

However, a continuing need exists for selective A_2 adenosine receptor agonists useful for therapeutic applications, that have reduced side effects.

6

Summary of the Invention

The present invention comprises compounds and methods of their use for the treatment of inflammatory activity in mammalian tissue. The inflammatory tissue activity can be due to pathological agents or can be due to physical,

5   chemical or thermal trauma, or the trauma of medical procedures, such as organ, tissue or cell transplantation, angioplasty (PCTA), inflammation following ischemia/reperfusion, or grafting. The present compounds comprise a novel class of 2-alkynyladenosine derivatives, substituted at the ethyne position by substituted cycloalkyl moieties. Preferably, the riboside residue is substituted at

10   the 5'-position ("X") by an N-alkyl-(or cycloalkyl)carboxyamino ("aminocarbonyl") moiety. Thus, the present invention provides a method for inhibiting the inflammatory response in a mammal, such as a human subject, and protecting the tissue subject to the response, by administering an effective amount of one or more compounds of the invention.

15   The compounds of the invention have the following general formula (I):

(I)

wherein (a) each R is individually hydrogen, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, phenyl or phenyl(C_1-C_3)-alkyl;

(b) X is -CH_2OH, -CO_2R^2, -$OC(O)R^2$ or $C(O)NR^3R^4$;

(c) each of R^2, R^3 and R^4 is individually H, C_{1-6}-alkyl; C_{1-6}-alkyl

20   substituted with 1-3 C_{1-6}-alkoxy, C_3-C_7 cycloalkyl, C_{1-6}-alkylthio, halogen, hydroxy, amino, mono(C_{1-6}-alkyl)amino, di(C_{1-6}-alkyl)amino, or C_{6-10}-aryl, wherein aryl may be substituted with 1-3 halogen, C_{1-6}-alkyl, hydroxy, amino, mono(C_{1-6}-alkyl)amino, or di(C_{1-6}-alkyl)amino; C_{6-10}-aryl; or C_{6-10}-aryl substituted with 1-3 halogen, hydroxy, amino, mono(C_{1-6}-alkyl)amino, di(C_{1-6}-

25   alkyl)amino, or C_{1-6}-alkyl;

7

(d) R^1 is $(X-(Z)-)_n[(C_3-C_{10})$cycloalkyl]-(Z')- wherein Z and Z' are individually (C_1-C_6)alkyl, optionally interrupted by 1-3 S or nonperoxide O, or is absent, and n is 1-3; or a pharmaceutically acceptable salt thereof.

The invention provides a compound of formula I for use in medical
5   therapy, preferably for use in treating or protecting tissue from inflammation such as an inflammatory response, as well as the use of a compound of formula I for the manufacture of a medicament for the treatment of an inflammatory response due to a pathological condition or symptom in a mammal, such as a human, which is associated with inflammation.

10   Although certain A_{2A} adenosine receptor agonists have been reported to be vasodilators, and thus to be useful to directly treat hypertension, thrombus, atherosclerosis and the like, the tissue-protective activity of the compounds of formula (I) is not suggested by the prior art.

The invention also includes the use of a combination of these compounds
15   with type IV phosphodiesterase inhibitors for synergistic decreases in the inflammatory response of immune cells.

The invention also provides a pharmaceutical composition comprising an effective amount of the compound of formula I, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable diluent or carrier,
20   and optionally, in combination with a Type IV phosphodiesterase (PDE) inhibitor. Preferably, the composition is presented as a unit dosage form.

Additionally, the invention provides a therapeutic method for preventing or treating a pathological condition or symptom in a mammal, such as a human, wherein the activity of A_{2A} adenosine receptors is implicated and agonism of said
25   activity is desired, comprising administering to a mammal in need of such therapy, an effective amount of a compound of formula I, or a pharmaceutically acceptable salt thereof. It is believed that activation of A_{2A} adenosine receptors inhibits inflammation by effecting neutrophils, mast cells, monocytes/macrophages, T-cells and/or eosinophils. Inhibition of these
30   inflammatory cells results in tissue protection following tissue insults.

Among the inflammatory responses that can be treated (including treated prophylactically) with a compound of formula I, optionally with a Type IV PDE inhibitor, are inflammation due to

8

(a) autoimmune stimulation (autoimmune diseases), such as lupus erythematosus, multiple sclerosis, infertility from endometriosis, type I diabetes mellitus including the destruction of pancreatic islets leading to diabetes and the inflammatory consequences of diabetes, including leg ulcers, Crohn's disease,

5   ulcerative colitis, inflammatory bowel disease, osteoporosis and rheumatoid arthritis;

(b) allergic diseases such as asthma, hay fever, rhinitis, vernal conjunctivitis and other eosinophil-mediated conditions;

(c) skin diseases such as psoriasis, contact dermatitis, eczema, infectious

10   skin ulcers, open wounds, cellulitis;

(d) infectious diseases including sepsis, septic shock, encephalitis, infectious arthritis, endotoxic shock, gram negative shock, Jarisch-Herxheimer reaction, shingles, toxic shock, cerebral malaria, bacterial meningitis, acute respiratory distress syndrome (ARDS), lyme disease, HIV infection, (TNFα-

15   enhanced HIV replication, TNFα inhibition of reverse transcriptase inhibitor activity);

(e) wasting diseases: cachexia secondary to cancer and HIV;

(f) organ, tissue or cell transplantation (e.g., bone marrow, cornea, kidney, lung, liver, heart, skin, pancreatic islets) including transplant rejection,

20   and graft versus host disease;

(g) adverse effects from drug therapy, including adverse effects from amphotericin B treatment, adverse effects from immunosuppressive therapy, e.g., interleukin-2 treatment, adverse effects from OKT3 treatment, adverse effects from GM-CSF treatment, adverse effects of cyclosporine treatment, and adverse

25   effects of aminoglycoside treatment, stomatitis and mucositis due to immunosuppression;

(h) cardiovascular conditions including circulatory diseases induced or exasperated by an inflammatory response, such as ischemia, atherosclerosis, peripheral vascular disease, restenosis following angioplasty, inflammatory

30   aortic aneurysm, vasculitis, stroke, spinal cord injury, congestive heart failure, hemorrhagic shock, ischemia/reperfusion injury, vasospasm following subarachnoid hemorrhage, vasospasm following cerebrovascular accident, pleuritis, pericarditis, and the cardiovascular complications of diabetes;

9

(i) dialysis, including pericarditis, due to peritoneal dialysis;

(j) gout; and

(k) chemical or thermal trauma due to burns, acid, alkali and the like.

Of particular interest and efficacy is the use of the present compounds to

5   treat inflammatory responses due to organ, tissue or cell transplantation, i.e., the transplantation of allogeneic or xenogeneic tissue into a mammalian recipient, autoimmune diseases and inflammatory conditions due to circulatory pathologies and the treatment thereof, including angioplasty, stent placement, shunt placement or grafting. Unexpectedly, it was found that administration of one or

10   more compounds of formula (I) was effective after the onset of the inflammatory response, e.g., after the subject was afflicted with the pathology or trauma that initiates the inflammatory response.

The invention also includes a method for measuring the response, or binding a compound of formula I at or to designated A_{2A} adenosine receptor sites

15   comprising said receptors, *in vivo* or *in vitro*, with an amount of a compound of formula I effective to bind to said receptors. Tissue or cells comprising ligand-bound receptor sites can be used to measure the selectively of test compounds for specific receptor subtypes, the amount of bioactive compound in blood or other physiological fluids, or can be used as a tool to identify potential therapeutic

20   agents for the treatment of diseases or conditions associated with receptor site activation, by contacting said agents with said ligand-receptor complexes, and measuring the extent of displacement of the ligand and/or binding of the agent, or the cellular response to said agent (e.g., cAMP accumulation).

Detailed Description of the Invention

25   The following definitions are used, unless otherwise described. Halo is fluoro, chloro, bromo, or iodo. Alkyl, alkoxy, aralkyl, alkylaryl, etc. denote both straight and branched alkyl groups; but reference to an individual radical such as "propyl" embraces only the straight chain radical, a branched chain isomer such as "isopropyl" being specifically referred to. Aryl includes a phenyl radical or

30   an ortho-fused bicyclic carbocyclic radical having about nine to ten ring atoms in which at least one ring is aromatic. Heteroaryl encompasses a radical attached via a ring carbon of a monocyclic aromatic ring containing five or six ring atoms consisting of carbon and one to four heteroatoms each selected from the group

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consisting of non-peroxide oxygen, sulfur, and N(X) wherein X is absent or is H, O, (C_1-C_4)alkyl, phenyl or benzyl, as well as a radical of an ortho-fused bicyclic heterocycle of about eight to ten ring atoms derived therefrom, particularly a benz-derivative or one derived by fusing a propylene, trimethylene, or

5   tetramethylene diradical thereto.

It will be appreciated by those skilled in the art that the compounds of formula (I) have more than one chiral center and may be isolated in optically active and racemic forms. Preferably, the riboside moiety of formula (I) is derived from D-ribose, i.e., the 3',4'-hydroxyl groups are alpha to the sugar ring

10   and the 2' and 5' groups is beta (3R, 4S, 2R, 5S). When the two groups on the cyclohexyl group are in the 4-position, they are preferably trans. Some compounds may exhibit polymorphism. It is to be understood that the present invention encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the invention, which

15   possess the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, or enzymatic techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase) and how to determine adenosine

20   agonist activity using the tests described herein, or using other similar tests which are well known in the art.

Specific and preferred values listed below for radicals, substituents, and ranges, are for illustration only; they do not exclude other defined values or other values within defined ranges for the radicals and substituents.

25   Specifically, (C_1-C_6)alkyl can be methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec-butyl, pentyl, 3-pentyl, or hexyl. As used herein, the term "cycloalkyl" encompasses bycycloalkyl (norbornyl, 2.2.2-bicyclooctyl, etc.) and tricycloalkyl (adamantyl, etc.), optionally comprising 1-2 N, O or S. Cycloalkyl also encompasses (cycloalkyl)alkyl. Thus, (C_3-C_6)cycloalkyl can be

30   cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl; (C_3-C_6)cycloalkyl(C_1-C_6)alkyl can be cyclopropylmethyl, cyclobutylmethyl, cyclopentylmethyl, cyclohexylmethyl;, 2-cyclopropylethyl, 2-cyclobutylethyl, 2-cyclopentylethyl, or 2-cyclohexylethyl.

11

(C_1-C_6)alkoxy can be methoxy, ethoxy, propoxy, isopropoxy, butoxy, iso-butoxy, sec-butoxy, pentoxy, 3-pentoxy, or hexyloxy; (C_2-C_6)alkenyl can be vinyl, allyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl, 1-pentenyl, 2-pentenyl, 3-pentenyl, 4-pentenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 4-hexenyl,

5   or 5-hexenyl; (C_2-C_6)alkynyl can be ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 3-butynyl, 1-pentynyl, 2-pentynyl, 3-pentynyl, 4-pentynyl, 1-hexynyl, 2-hexynyl, 3-hexynyl, 4-hexynyl, or 5-hexynyl; (C_1-C_6)alkanoyl can be acetyl, propanoyl or butanoyl; halo(C_1-C_6)alkyl can be iodomethyl, bromomethyl, chloromethyl, fluoromethyl, trifluoromethyl, 2-chloroethyl, 2-fluoroethyl, 2,2,2-

10   trifluoroethyl, or pentafluoroethyl; hydroxy(C_1-C_6)alkyl can be hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, 1-hydroxybutyl, 4-hydroxybutyl, 1-hydroxypentyl, 5-hydroxypentyl, 1-hydroxyhexyl, or 6-hydroxyhexyl; (C_1-C_6)alkoxycarbonyl (CO_2R^2) can be methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl,

15   isopropoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, or hexyloxycarbonyl; (C_1-C_6)alkylthio can be methylthio, ethylthio, propylthio, isopropylthio, butylthio, isobutylthio, pentylthio, or hexylthio; (C_2-C_6)alkanoyloxy can be acetoxy, propanoyloxy, butanoyloxy, isobutanoyloxy, pentanoyloxy, or hexanoyloxy; aryl can be phenyl, indenyl, or naphthyl; and heteroaryl can be

20   furyl, imidazolyl, triazolyl, triazinyl, oxazoyl, isoxazoyl, thiazolyl, isothiazoyl, pyraxolyl, pyrrolyl, pyrazinyl, tetrazolyl, puridyl (or its N-oxide), thientyl, pyrimidinyl (or its N-oxide), indolyl, isoquinolyl (or its N-oxide) or quinolyl (or its N-oxide).

A specific value for R is amino, monomethylamino or cyclopropylamino.

25   A specific value for R^1 is carboxy- or (C_1-C_4)alkoxycarbonyl-cyclohexyl(C_1-C_4)alkyl.

A specific value for R^2 is H or (C_1-C_4)alkyl, i.e., methyl or ethyl.

A specific value for R^3 is H, methyl or phenyl.

A specific value for R^4 is H, methyl or phenyl.

30   A specific value for Z is -CH_2- or -CH_2-CH_2-.

A specific value for X is CO_2R^2, (C_2-C_5)alkanoylmethyl or amido.

A specific value for n is 1.

12

Preferred compounds of formula (I) are those wherein each R is H, X is ethylaminocarbonyl and R^1 is 4-carboxycyclohexylmethyl (DWH-146a), R^1 is 4-methoxycarbonylcyclohexylmethyl (DWH-146e) or R^1 is 4-acetoxymethyl-cyclohexylmethyl (JMR-193). They are depicted below (DWH-146 (acid) and

5   methylester (e)) and JMR-193.

DWH-146 (acid, X=H; ester, X=Me)

JMR193

5
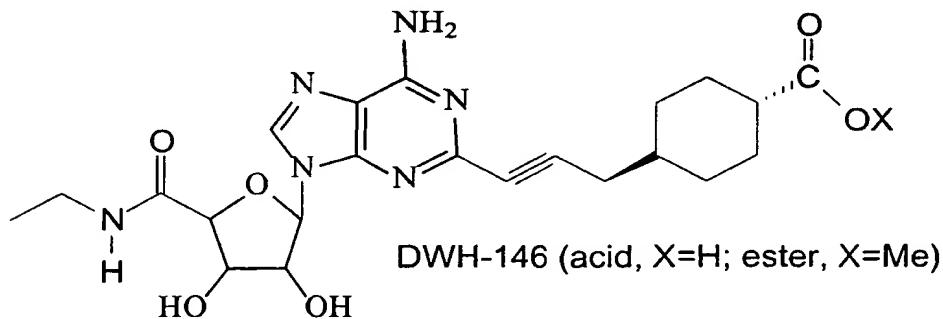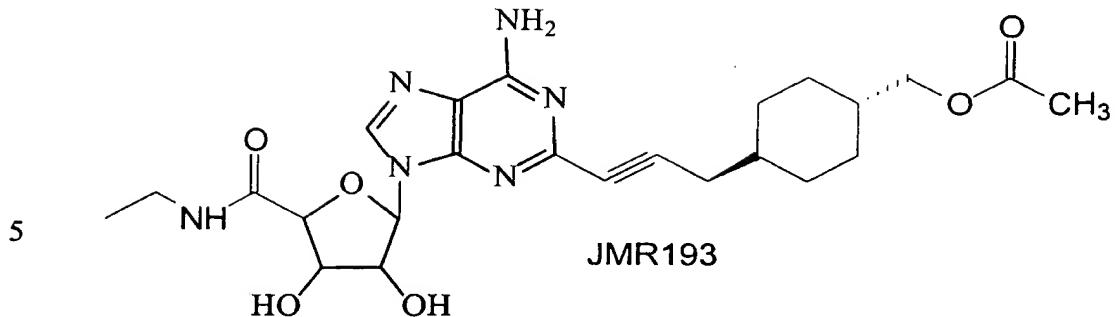
The synthesis of methyl 4[3-(6-amino-9(5-[(ethylamino)carbonyl]-3,4-dihydroxytetrahydro-Z-furanyl-9*H*-2-purinyl)-2-propynyl]-

10   1-cyclohexanecarboxylate (DWH-146e) was accomplished by the cross coupling of an iodo-adenosine derivative (N-ethyl-1'-deoxy-1'-(amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuoramide) with methyl 4-(2-propynyl)-1-cyclohexanecarboxylate by utilization of a Pd[II] catalyst. The synthesis of the iodo-adenosine derivative was accomplished from guanosine. Guanosine is first

15   treated with acetic anhydride, which acetalates the sugar hydroxyls, followed by the chlorination of position 6 with tetramethyl ammonium chloride and phosphorousoxychloride. Iodination of position 2 was accomplished via a modified Sandmeyer reaction, followed by displacement of the 6-Cl and sugar acetates with ammonia. The 2' and 3' hydroxyls were protected as the acetonide

20   and the 5' hydroxyl was iodized to the acid with potassium permanganate. Deptrotection of the 2' and 3' acetonide, Fisher esterification of the 5' acid with ethanol and conversion of the resulting ethyl ester to the ethyl amide with ethylamine gave N-ethyl-1'-deoxy-1'-(amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuoramide.

25   The acetylene (methyl 4-(2-propynyl)-1-cyclohexanecarboxylate) was synthesized starting from *trans*-1,4-cyclohexanedimethanol. Initially the *trans*-diol was monotosylated followed by displacement of the tosylate with an acetylene anion. The hydroxyl of the resulting hydroxyl acetylene species was oxidized to the acid via Jones reagent followed by methylation with

30   (trimethylsilyl)diazomethane to give methyl 4-(2-propynyl)-1-cyclohexanecarboxylate.

The cross-coupling reaction was performed under the following previously reported conditions. To a solution of *N,N*-dimethylformamide (0.5

14

mL), acetonitrile (1 mL), triethylamine (0.25 mL), and *N*-ethyl-1'-deoxy-1'-(amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuroamide (25 mg, 0.06 mmol) was added bis(triphenylphosphine)palladium dichloride (1 mg, 2 mol%) and copper(I)iodide (0.06 mg, .5 mol%). To the resulting mixture was added methyl

5   4-(2-propynyl)-1-cyclohexanecarboxylate (54 mg, 0.3 mmol) and the reaction was stirred under N_2 atmosphere for 16 hours. The solvent was removed under vacuum and the resulting residue was flash chromatographed in 20% methanol in chloroform (R_f = 0.45) to give 19 mg (off-white solid, mp 125°C (decomposed)) of 4[3-(6-amino-9(5-[(ethylamino)carbonyl]-3,4-

10   dihydroxytetrahydro-Z-furanyl)-9*H*-2-purinyl)-2-propynyl]-1-cyclohexanecarboxylate (DWH-146e).

DWH-146e and JMR193 are substantially more potent as inhibitors in inflammatory model systems than the reference compound, CGS21680 (2-[*p*-(carboxyethyl)-phenyl-ethylamino]5'-N-ethylcarboxamidoadenosine). For

15   example, DWH-146e is about 80 times more potent at A_{2A} receptors and 40 times more selective for A_{2A} over A_3 receptors than is CGS21680.

Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, malate, acetate, citrate, malonate, tartarate,

20   succinate, benzoate, ascorbate, α-ketoglutarate, and α-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

Pharmaceutically acceptable salts may be obtained using standard procedures well known in the art, for example by reacting a sufficiently basic

25   compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

The compounds of formula I can be formulated as pharmaceutical

30   compositions and administered to a mammalian host, such as a human patient in a variety of forms adapted to the chosen route of administration, i.e., orally or parenterally, by intravenous, intramuscular, topical or subcutaneous routes.

15

Thus, the present compounds may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be

5   incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The

10   percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

The tablets, troches, pills, capsules, and the like may also contain the

15   following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring

20   may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and

25   the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active

30   compound may be incorporated into sustained-release preparations and devices.

The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts can be prepared in water, optionally mixed with a nontoxic surfactant.

16

Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

5   The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable

10   under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the

15   formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for

20   example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the

25   other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

30   For topical administration, the present compounds may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

17

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or water-alcohol/glycol blends, in which the present compounds can be dissolved or dispersed at effective levels, optionally

5   with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

10   Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Examples of useful dermatological compositions which can be used to

15   deliver the compounds of formula I to the skin are disclosed in Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508).

Useful dosages of the compounds of formula I can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods

20   for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949. Useful dosages of Type IV PDE inhibitors are known to the art. For example, see, U.S. Pat. No. 5,877,180, Col. 12.

Generally, the concentration of the compound(s) of formula (I) in a liquid

25   composition, such as a lotion, will be from about 0.1-25% wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected

30   but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

18

In general, however, a suitable dose will be in the range of from about 0.5 to about 100 μg/kg, e.g., from about 10 to about 75 μg/kg of body weight per day, such as 3 to about 50 μg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 μg/kg/day, most preferably in the range of 15

5   to 60 μg/kg/day.

The compound is conveniently administered in unit dosage form; for example, containing 5 to 1000 μg, conveniently 10 to 750 μg, most conveniently, 50 to 500 μg of active ingredient per unit dosage form.

Ideally, the active ingredient should be administered to achieve peak

10   plasma concentrations of the active compound of from about 0.1 to about 10 nM, preferably, about 0.2 to 10 nM, most preferably, about 0.5 to about 5 nM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 μg of the active ingredient. Desirable blood levels

15   may be maintained by continuous infusion to provide about 0.01-5.0 μg/kg/hr or by intermittent infusions containing about 0.4-15 μg/kg of the active ingredient(s).

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three,

20   four or more sub-doses per day. The sub-dose itself may be further divided, e.g., into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator or by application of a plurality of drops into the eye. For example, it is desirable to administer the present compositions intravenously over an extended period of time following the insult that gives rise

25   to inflammation.

The ability of a given compound of the invention to act as an A_{2A} adenosine receptor agonist (or antagonist) may be determined using pharmacological models which are well known to the art, or using tests described below.

30   The invention will be further described by reference to the following detailed examples, which are given for illustration of the invention, and are not intended to be limiting thereof.

**Example 1. *Trans*-(1-[4-hydroxymethyl)cyclohexyl]methyl)-4-
methylbenzenesulfonate (5.2).** Sodium hydride (1.68 g, 70 mmol) was added
to a solution of 10 g (70 mmol) [4-(hydroxymethyl)cyclohexyl]methan-1-ol **(5.1)**
in 700 mL of tetrahydrofuran and stirred for 1 hour p-toluenesulfonyl chloride

5   (13.3 g, 70 mmol) was then added and the reaction mixture was refluxed for 5
hours. The reaction was then cooled to 0°C and slowly quenched with water
until there is no more reactive hydride. Once the hydride was quenched, the
reaction mixture was diluted with ether (700 mL) and extracted 2 times with
10% aqueous potassium carbonate (700 mL). The organics were dried using

10   sodium sulfate and the solvent was removed under reduced pressure. The
product was purified by chromatography on silica gel column eluting with
acetone-dichloromethane (5:95) to give **5.2** (35%). ^{1}H NMR (300 MHz, CDCl$_3$)
δ 7.75 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.1 Hz, 2H), 3.79 (d, J = 6.35 Hz, 2H),
3.39 (d, J = 6.35 Hz, 2H), 2.42 (s, 3H), 1.75 (m, 4H), 1.59 (m, 1H), 1.37 (m,

15   1H), 0.9 (m, 4H). ^{13}C NMR (300 MHz, CDCl$_3$) δ 145.3, 133.4, 130.3, 130.3,
128.3, 128.3, 75.8, 68.5, 40.6, 37.8, 28.9, 28.9, 28.9, 28.9, 22.1.

Example 2. (4-prop-2-ynylcyclohexyl)methan-1-ol (5.3). Lithium
acetylide ethylenediamine complex (90%) (6.4 g, 70 mmol) was added very
slowly to a solution of **5.2** (3 g, 10 mmol) in 40 mL of dimethylsulfoxide. The

20   reaction mixture was allowed to stir for 5 days and then slowly quenched at 0°C
with water. This mixture was diluted with ether (300 mL) and extracted 3 times
with saturated aqueous ammonium chloride (200 mL). The organics were dried
with sodium sulfate. The solvent was removed under reduced pressure and the
product was purified by chromatography on silica gel column eluting with ethyl

25   acetate-hexanes (20:80) to give **5.3** (85%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 3.41
(d, J = 6.5 Hz, 2H), 2.07 (dd, J = 2.5, 6.5 Hz, 2H), 1.96-1.75 (m, 5H), 1.41 (m,
2H), .095 (m, 4). ^{13}C NMR (300 MHz, CDCl$_3$) δ 83.8, 69.6, 68.9, 40.7, 37.7,
32.3, 32.3, 29.6, 29.6, 26.5.

Example 3. 4-prop-2-ynylcyclohexanecarboxylic acid (5.4). A

30   solution of chromium trioxide (1.1 g, 11 mmol) in 1.5 M sulfuric acid (40 mL,
27 mmol) was maintained at 0°C while **5.3** (0.46 g, 3 mmol) in 80 mL of acetone
was added over 2 hours. The reaction was then stirred for an additional 2 hours
at room temperature. The reaction mixture was diluted with ether (200 mL) and

20

extracted 2 times with water. The organics were dried with sodium sulfate. The
solvent was removed under reduced pressure and the product was purified by
chromatography on silica gel column eluting with acetone-dichloromethane
(70:30) to give **5.4** (75%). ^{1}H NMR (300 MHz, CDCl$_3$) δ 2.24 (dt, J = 3.66, 12.1
Hz, 1H), 2.10 (dd, J = 2.7, 6.5 Hz, 2H), 2.04-1.89 (m, 5H), 1.76 (d, J = 2.3 Hz,
1H), 1.43 (dq, J = 3.28, 13.1 Hz, 2H), 1.03 (dq, J = 3.28, 13.1 Hz, 2H). ^{13}C
NMR (300 MHz, CDCl$_3$) δ 183.2, 83.3, 69.9, 43.4, 36.7, 31.8, 28.9, 26.3.

Example 4. Methyl 4-prop-2-ynylcyclohexanecarboxylate (5.5).
(Trimethylsilyl)diazomethane (2.0 M) solution in hexanes (1 mL, 2 mmol) was
added to a solution of **5.4** (0.34 g, 2 mmol) in 15 mL of
methanol:dichloromethane (3:7). The solvents were removed under reduced
pressure resulting in 100% conversion of starting material to product. ^{1}H NMR
(300 MHz, CDCl$_3$) δ 2.24 (dt, J = 3.66, 12.1 Hz, 1H), 2.10 (dd, J = 2.7, 6.5 Hz,
2H), 2.06 (dd, J = 1.54, 6.54 Hz, 1H), 2.00-1.89 (m, 3H), 1.76 (d, J = 2.3 Hz,
1H), 1.43 (dq, J = 3.28, 13.1 Hz, 2H), 1.03 (dq, J = 3.28, 13.1 Hz, 2H). ^{13}C
NMR (300 MHz, CDCl$_3$) δ 176.8, 83.3, 69.8, 51.9, 43.4, 36.7, 31.9, 29.2, 26.3.

**Example 5. [(2R,3R,4R,5R)-3,4-diacetyloxy-5-(2-amino-6-
oxohyropurin-9-yl)oxolan-2-yl]methyl acetate (6.2).** A suspension of 113 g
(0.4 mol) of dry guanosine (**6.1**), acetic anhydride (240 mL, 2.5 mol), dry
pyridine (120 mL) and dry DMF (320 mL) was heated for 3.75 hours at 75°C
without allowing the temperature to exceed 80°C. The clear solution was then
transferred to a 3L Erlenmyer flask and filled with 2-propanol. Upon cooling the
solution to room temperature crystallization was initiated and allowed to proceed
at 4°C overnight. The white solid filtrate was filtered, washed with 2-propanol
and recrystallized from 2-propanol to give **6.2** (96%). ^{1}H NMR (300 Mhz,
CDCl$_3$) δ 8.20 (s, 1H, H-8), 6.17 (d, J = 5.41 Hz, 1 H, H-1') 5.75 (t, J = 5.39 Hz,
1H, H-2'), 5.56 (t, J = 5.0, H-3'), 4.41 (m, 3H, H-4',5'), 2.14 (s, 3H, Ac), 2.11 (s,
3H, Ac), 2.10 (s, 3H, Ac). ^{13}C NMR (300 MHz, CD$_3$OD) δ 171.0, 170.3, 1702,
157.7, 154.8, 152.4, 136.7, 117.7, 85.5, 80.4, 73.0, 71.3, 64.0, 31.3, 21.2, 21.0.

**Example 6. [(2R,3R,4R,5R)-3,4-diacetyloxy-5-(2-amino-6-
chloropurin-9-yl)oxolan-2-yl]methyl acetate (6.3).** To a 1000 mL flask was
added 80 g (0.195 mol) [(2R,3R,4R,5R)-3-4-diacetyloxy-5-(2-amino-6-
oxohyropurin-9-yl)oxolan-2-yl]methyl acetate (**6.2**), tetramethylammonium

21

chloride (44 g, 0.4 mol), anhydrous acetonitrile (400 mL) and N,N-dimethlaniline (25 mL). The flask was placed in an ice salt bath and cooled to 2°C. To this solution was added dropwise POCl$_3$ (107 mL 1.15 mol) at a rate that maintained the temperature below 5°C (45 minutes). The flask was then

5   removed from the ice bath, outfitted with a condenser, placed in an oil bath and allowed to reflux for 10 minutes whereas the solution changed to a red/brown color. The solvent was then removed under reduced pressure to yield an oily residue which was transferred to a beaker containing 1000 g of ice and 400 mL of CHCl$_3$ and allowed to stir for 1.5 hours to decompose any remaining POCl$_3$.

10   The organic phase was then removed and the aqueous phase extracted with 3 × 50 mL of CHCl$_3$ and pooled with the organic phase. The pooled organic was then back extracted with 50 mL of water followed by stirring with 200 mL of saturated NaHCO$_3$. The organic was further extracted with NaHCO$_3$ until the aqueous extract was neutral (2X). The organic was finally extracted with brine

15   and then dried over MgSO$_4$ for 16 hours. To the solution was added 800 mL of 2-propanol after which the solution was concentrated under reduced pressure. To the oily solid was added 200 mL of 2-propanol and the solution was refrigerated overnight. The crystalline product was filtered, washed, and allowed to dry overnight to give 6.3 (77%). ^{1}H NMR (300 MHz, CD$_3$OD) δ 8.31 (s, 1H,

20   H-8), 7.00 (s, 2H, NH$_2$) 6.06 (d, J = 5.8 Hz, 1H, H-1′), 5.83 (t, J = 6.16 Hz, 1H, H-2′), 5.67 (m, 1H, H-3′), 4.29 (m, 3H, H-4′,5′), 2.07 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.98 (s, 3H, Ac). ^{13}C NMR (300 MHz, CD$_3$OD) δ 171.0, 170.4, 170.2, 160.8, 154.6, 150.8, 142.2, 124.5, 85.8, 80.6, 72.8, 71.2, 63.9, 21.4, 21.3, 21.1.

Example 7. [(2R,3R,4R,5R)-3,4-diacetyloxy-5-(6-chloro-2-iodopurin-

25   **9-yl)oxolan-2-yl]methyl acetate (6.4).** Isoamyl nitrite (5 mL, 37 mmol) was added to a mixture of 5.12 g (12 mmol) [(2R,3R,4R,5R)-3-,4-diacetyloxy-5-(2-amino-6-chloropurin-9-yl)oxolan-2-yl]methyl acetate (6.3), I$_2$ (3.04 g, 12 mmol), CH$_2$I$_2$ (10 mL, 124 mmol), and CuI (2.4 g, 12.6 mmol) in THF (60 mL). The mixture was heated under reflux for 45 minutes and then allowed to cool to room

30   temperature. To this solution was added 100 ml of sat. Na$_2$S$_2$O$_3$ which removed the reddish color due to iodine. The aqueous was extracted 3X with chloroform, which was pooled, dried over MgSO$_4$, and concentrated under reduced pressure. The product was then purified over a silica gel column using CHCl$_3$-MeOH

22

(98:2) to collect [(2R,3R,4R,5R)-3,4-diacetyloxy-5-(6-chloro-2-iodopurin-9-yl)oxolan-2-yl]methyl acetate (6.4) (80% crystallized from EtOH). ¹H NMR (300 MHz, CDCl₃) δ 8.20 (s, 1H H-8), 6.17 (d, J = 5.41 Hz, 1H, H-1′), 5.75 (t, J = 5.39 Hz, 1H, H-2′), 5.56 (t, J = 5.40 Hz, 1H, H-3′), 4.38 (m, 3H, H-4′,5′), 2.14

5   (s, 1H, Ac), 2.11 (s, 1H, Ac), 2.10 (s, 1H, Ac).

Example 8. (4S,2R,3R,5R)-2-(6-amino-2-iodopurin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol (6.5). To a flask containing 6.0 g (11.1 mmol) [(2R,3R,4R,5R)-3,4-diacetyloxy-5-(6-chloro-2-iodopurin-9-yl)oxolan-2-yl]methyl acetate (6.4) was added 100 ml of liquid NH₃ at -78°C and the solution

10   was allowed to stir for 6 hours. After which time it was allowed to come to r.t. overnight with concurrent evaporation of the NH₃ to yield a brown oil. The product was crystallized from hot isopropanol to give 6.5 (80%), m.p. 143-145°C, r.f. = 0.6 in 20% MeOH/CHCl₃. ¹H NMR (300 MHz, DMSO-d₆) δ 8.24 (s, 1H), 7.68 (s, 2H), 5.75 (d, J = 6.16, 1H), 5.42 (d, J = 5.40 Hz, 1H), 5.16 (d, J

15   = 4.62 Hz, 1H), 4.99 (t, J = 5.39 Hz, 1H), 4.67 (d, J = 4.81 Hz, 1H), 4.06 (d, J = 3.37 Hz, 1H), 3.89 (m, 1H), 3.54 (m, 2H).

Example 9. [(1R,2R,4R,5R)-4-(6-amino-2-iodopurin-9-yl)-7-7-dimethyl-3,6,8-trioxabicyclo[3.3.0]oct-2-yl]methan-1-ol (6.6). To a solution of 2.0 g (5.08 mmol) (4S,2R,3R,5R)-2-(6-amino-2-iodopurin-9-yl)-

20   5(hydroxymethyl)oxolane-3,4-diol (6.6) in 100 mL acetone was added 9.6 g of p-toluenesulfonic acid and 5 ml of dimethoxypropane. The reaction was stirred at room temperature for 1 hour at which time 15 g of NaHCO₃ and then stirred for an additional 3 hours. the residue was filtered and washed 2X with EtOAc. The filtrate was then concentrated under reduced pressure. The residue was

25   chromatographed on a silica gel column with MeOH-CHCl₃ (1:99) to give 6.6 (72%) as a solid, m.p. 185-187°C. ¹H NMR (300 MHz, DMSO-d₆) δ 8.22 (s, 1H, H-8), 7.69 (s, 2H, NH₂), 6.00 (d, J = 2.70 Hz, 1H, H-1′), 5.21 (m, 1H, H-2′), 5.07 (bs, 1H, OH), 4.88 (m, 1H, H-3′), 4.13 (m, 1H, H-4′), 3.47 (m, 2H, H-5′), 1.49 and 1.28 (s, 3H, C(CH₃)₂).

30   Example 10. (2S,1R,4R,5R)-4-(6-amino-2-iodopurin-9-yl)-7,7-dimethyl-3,6,8-trioxabicyclo[3.3.0]octane-2-carboxylic acid (6.7). To a stirred solution of 1.6 g (3.7 mmol) of [(1R,2R,4R,5R)-4-(6-amino-2-iodopurin-9-yl)-7-7-dimethyl-3,6,8-trioxabicyclo[3.3.0]oct-2-yl]methan-1-ol (6.6) in 200

23

mL of H_2O was added 0.60 g of KOH and, dropwise, a solution of 1.70 g (10.8 mml) of $KMnO_4$ in 50 mL of H_2O. The mixture was set aside in the dark at room temperature for 225 hours. The reaction mixture was then cooled to 5-10°C and decolorized by a solution of 4 mL of 30% H_2O2 in 16 mL of water,

5   while the temperature was maintained under 10°C using an ice-salt bath. The mixture was filtered through Celite and the filtrate was concentrated under reduced pressure to about 10 mL and then acidified to pH 4 with 2N HCl. The resulting precipitate was filtered off and washed with ether to yield **6.7** (70%) after drying as a white solid, m.p. 187-190°C. ^1H NMR (300 MHz, DMSO-d_6) δ

10   8.11 (s, 1H, H-8), 7.62 (s, 2H, NH_2), 7.46 (s, 1H, COOH), 6.22 (s, 1H, H-1′), 5.42 (d, J = 5.71 Hz, 1H, H-2′), 5.34 (d, J = 6.16 Hz, 1H, H-3′), 4.63 (s, 1H, H-4′), 1.46 and 1.30 (s, 3H, $C(CH_3)_2$).

Example 11. (2S,3S,4R,5R)-5-(6-amino-2-iodopurin-9-yl)-3,4-dihydroxyoxolane-2-carboxylic acid (6.8). A solution of 1.72 g (3.85 mmol)

15   of (2S,1R,4R,5R)-4-(6-amino-2-iodopurin-9-yl)-7,7-dimethyl-3,6,8-trioxabicyclo[3.3.0]octane-2-carboxylic acid (6.7) in 80 mL of 50% HCOOH was stirred at 80°C for 1.5 hours. The reaction mixture was evaporated under reduced pressure, dissolved in H_2O, and the solution was evaporated again. This process was repeated until there was no odor of formic acid in the residue.

20   Recrystallization from water yielded 1.33 g (85%) **6.8** as a white solid, m.p. 221-223°C, dec. ^1H NMR (300 MHz, DMSO-d_6) δ 8.31 (s, 1H, H-8), 7.68 (s, 2H, NH_2), 5.90 (d, J = 6.55 Hz, 1H, H-1′), 4.42 (m, 1H, H-2′), 4.35 (d, J = 2.31 Hz, 1H, H-4′), 4.22 (m, 1H, H-3′).

Example 12. [(2S,3S,4R,5R)-5-(6-amino-2-iodopurin-9-yl)-3,4-

25   **dihydroxyoxolan-2-yl]-N-ethylcarboxamide (6.9).** To a cooled (5°C) and stirred solution of 1.29 g (3.17 mmol) of (2S,3S,4R,5R)-5-(6-amino-2-iodopurin-9-yl)-3,4-dihydroxyoxolane-2-carboxylic acid (6.8) in 150 mL of absolute ethanol was added dropwise 1.15 mL of ice-cooled $SOCl_2$. The mixture was stirred at room temperature overnight and then brought to pH 8 with saturated

30   aqueous $NaHCO_3$. The mixture was filtered, and then the filtrate was concentrated under reduced pressure to yield a white solid which was dried and then redissolved in 20 mL of dry ethylamine at -20°C for 3 hours and then at room temperature overnight. The reaction mixture was diluted with absolute

24

ethanol, and the precipitated product was filtered off and washed with dry ether
to give 530 mg (72%) of **6.9** as a pure solid, m.p. 232-234°C. ^{1}H NMR (300
MHz, DMSO-d$_6$) δ 8.34 (s, 1H, H-8), 8.12 (t, 1H, NH), 7.73 (s, 2H, NH$_2$), 5.85,
(d, J = 6.93 Hz, 1H, H-1'), 4.54 (m, 1H, H-2'), 4.25 (d, J = 1.92 Hz, 1H, H-4'),
5   4.13 (m, 1H, H-3'), 3.28 (m, 2H, CH$_2$CH$_3$), 1.00 (t, J = 7.2 Hz, 3H, CH$_2$CH$_3$).

**Example 13. Methyl-4-(3-{9-[(4S,5S,2R,3R)-5-(N-ethylcarbamoyl)-
3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl)}prop-2-ynyl)cyclohexane-
carboxylate (DWH-146e).** To a degassed solution of 25 mg (0.063 mmol) of
[(2S,3S,4R,5R)-5-(6-amino-2-iodopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]-N-
10   ethylcarboxamide **(6.9)**, 16.9 mg (0.094 mmol) **(5.5)**, and 0.75 mg CuI in 5 mL
each of triethyl amine (TEA) and acetonitrile was added 15 mg of Pd(PPh$_3$)$_4$.
The solution was stirred for 24 hours at 70°C after which time the solution was
filtered through celite and chromatographed on silica gel with MeOH-CHCl$_3$
(5:95) to give **DWH-146e (24%).**

15   **Example 14. (4-prop-2-ynylcyclohexyl)methyl acetate (5.6).** Acetic
anhydride (0.92 mL, 8.25 mmol) and pyridine (.2 mL, 2.5 mmol) were added to
a solution of 5.3 (250 mg, 1.65 mmol) in 25 mL ether. The reaction was allowed
to stir at ambient temperature for 24 hours. Water was added to the reaction and
the organic was further extracted with 10% NaHCO$_3$. The organic layer was
20   dried with MgSO$_4$ and evaporated. The residue was chromatographed on silica
gel with EtOAc-Hexanes (5:95) to yield 5.6 (47%).

**Example 15. [4-(3-{9-(4S,5S,2R,3R)-5-(N-ethylcarbamoyl)-3,4-
dihydroxyoxolan-2-yl]-6-aminopurin-2-yl}prop-2-ynyl)cyclohexyl]methyl
acetate (JMR193).** To a degassed solution of 125 mg (0.29 mmol) of
25   [(2S,3S,4R,5R)-5-(6-amino-2-iodopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]-N-
ethylcarboxamide (6.9), 150 mg (0.77 mmol) (5.6), and 1.0 mg CuI in 1.3 mL of
TEA and 4 mL DMF was added to 25 mg of Pd(PPh$_3$)$_4$. The solution was stirred
for 72 hours at 60°C after which time the solution was filtered through celite and
chromatographed on silica gel with MeOH-CHCl$_3$ (5:95) to give JMR193 (10%).

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Example 16. [(2S,3S,4R,5R)-5-(6-amino-2-{3-[4-(hydroxymethyl)-cyclohexyl]prop-2-ynyl}purin-9-yl)-3,4-dihydroxyoxolan-2-yl]-N-ethyl-carboxamide.

A. (4-prop-2-ynylcyclohexyl)methan-1-ol. A lithium acetylide

5   ethylenediamine complex (90%) (6.4 g, 70 mmol) was added very slowly to a
solution of *trans*-[4-(hydroxymethyl)cyclohexyl]methyl 4-methylbenzene-
sulfonate (3 g, 10 mmol) in 40 mL of dimethylsulfoxide. The reaction mixture
was allowed to stir for 5 days and then slowly quenched at 0° C with water.
This mixture was diluted with ether (300 mL) and washed 3 times with saturated

10   aqueous ammonium chloride (200 mL). The organics were dried with sodium
sulfate. The solvent was removed under reduced pressure. The product was
purified by chromatography on silica gel column eluting with ethyl acetate-
hexanes (20:80) to furnish the product (85%). ^{1}H NMR (300 MHz, CDCl$_3$) d
3.41 (d, J = 6.5 Hz, 2H), 2.07 (dd, J = 2.5, 6.5 Hz, 2H), 1.96-1.75 (m, 5H), 1.41

15   (m, 2H), .095 (m, 4). ^{13}C NMR (300 MHz, CDCl$_3$) d 83.8, 69.6, 68.9, 40.7, 37.7,
32.3, 32.3, 29.6, 29.6, 26.5.

B. [(2S,3S,4R,5R)-5-(6-amino-2-{3-[4-(hydroxymethyl)cyclohexyl]-prop-1-ynyl}purin-9-yl)-3,4-dihydroxyoxolan-2-yl]-N-ethylcarboxamide (JMR2037). Pd(PPh$_3$)$_4$, 10 mg, was added to a degassed solution of 28mg

20   (0.065mmol) of [(2S,3S,4R,5R)-5-(6-amino-2-iodopurin-9-yl)-3,4-
dihydroxyoxolan-2-yl]-N-ethylcarboxamide, 30 mg (0.20 mmol) of (4-prop-2-
ynylcyclohexyl)methan-1-ol, and 1.0 mg CuI in 1 mL of triethyl amine (TEA), 1
mL DMF, and 1 mL acetonitrile. The solution was stirred for 60 hours at room
temperature after which time the solution was filtered through celite and

25   chromatographed on silica gel with MeOH-CHCl$_3$ (7:93) to give 5mg (17%)
JMR2037. The title compound was tested using the binding assays disclosed
herein and found to bind to recombinant human A$_{2A}$ receptors, Ki of 694 ± 69
nM.

Example 17. Radioligand Binding Studies. Binding to A$_{2A}$ receptors

30   was evaluated with the radioligand ^{125}I-ZM241385. Figure 2B depicts the
competition by selective agonists for binding to recombinant human A$_{2A}$
adenosine receptors. DWH-146e is highly selective for the recombinant human
A$_{2A}$ (hA2A) subtype. Selectivity for the A$_3$ receptor (not shown) is less

26

impressive, but still about 50-fold. DWH-146e is about 5 and 50 times more potent than WRC0470 and CGS21680, respectively (Fig. 1). An unexpected and interesting finding is that the ester, DWH-146e also is about 50 times more potent than the acid, DWH-146a (Fig. 1).

5   **Example 17A. Effect of DWH-146e and JMR193 on Neutrophil Oxidative Activity**

A. Materials.

f-met-leu-phe (fMLP), luminol, superoxide dismutase, cytochrome C, fibrinogen, adenosine deaminase, and trypan blue were obtained from Sigma

10   Chemical. Ficoll-hypaque was purchased from ICN (Aurora, OH), and Cardinal Scientific (Santa Fe, NM) and Accurate Chemicals and Scientific (Westerbury, NY). Endotoxin (lipopolysaccharide; *E. coli* K235) was from List Biologicals (Campbell, CA). Hanks balanced salt solution (HBSS), and limulus amebocyte lysate assay kit were from BioWittaker (Walkersville, MD). Human serum

15   albumin (HSA) was from Cutter Biological (Elkhart, IN). Recombinant human tumor necrosis factor-alpha was supplied by Dianippon Pharmaceutical Co. Ltd. (Osaka, Japan). ZM241385 (4-(2-[7-amino-2-(2-furyl)-[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)phenol) was a gift from Simon Poucher, Zeneca Pharmaceuticals, Cheshire, UK. Stock solutions (1 mM and 10 mM in DMSO)

20   were made and stored at -20°C.

B. Human neutrophil preparation

Purified neutrophils (~98% neutrophils and >95% viable by trypan blue exclusion) containing <1 platelet per 5 neutrophils and < 50 pg/ml endotoxin (limulus amebocyte lysate assay) were obtained from normal heparinized (10

25   U/ml) venous blood by a one step Ficoll-hypaque separation procedure (A. Ferrante et al., J. Immunol. Meth., 36, 109 (1980)).

C. Release of inflammatory reactive oxygen species from primed and stimulated human neutrophils Chemiluminescence

Luminol-enhanced chemiluminescence, a measure of neutrophil

30   oxidative activity, is dependent upon both superoxide production and mobilization of the lysosomal granule enzyme myeloperoxidase. The light is emitted from unstable high-energy oxygen species generated by activated neutrophils. Purified neutrophils (5-10 × 10^5/ml) were incubated in Hanks

27

balanced salt solution containing 0.1% human serum albumin (1 ml) with or without DWH-146a, DWH-146e, CGS21680, or JMR193 with or without rolipram and with or without tumor necrosis factor-alpha (1 U/ml) for 30 minutes at 37°C in a shaking water bath. Then luminol (1×10^{-4} M) enhanced f-

5   met-leu-phe (1 mcM) stimulated chemiluminescence was read with a Chronolog® Photometer (Crono-log Corp., Havertown, PA) at 37°C for 2-4 minutes. Chemiluminescence is reported as relative peak light emitted (= height of the curve) compared to samples with tumor necrosis factor-alpha and without DWH, JMR or rolipram.

10   D. Results

As shown in Figure 2, JMR193 and DWH-146e both decreased tumor necrosis factor-alpha-primed f-met-leu-phe-stimulated human neutrophil oxidative activity as measured by luminol-enhanced chemiluminescence more effectively than the adenosine A_{2A} receptor agonist CGS21680. The horizonal

15   axis gives the concentration of CGS21680, DWH-146a, DWH-146e or JMR193 (log nM). The vertical axis gives the resulting peak human neutrophil activity as relative amount of stimulated release of reactive oxygen species as measured with luminol-enhanced chemiluminescence compared to control samples which were not primed with tumor necrosis factor-alpha. Means SEM (n=4-5 separate

20   experiments).

The data below the horizontal axis in Fig. 2 gives the EC_{50} for reducing the human neutrophil activity (based on the data in Fig. 2). Means SEM (n = 4-5 separate experiments). *p < 0.05 decreased IC_{50} compared to CGS21680.

JMR193 and DWH-146e decreased the stimulated-neutrophil oxidative

25   burst with EC_{50}'s less than 1 nM (0.8 and 0.3 nM, respectively). In contrast, the free acid A_{2A} adenosine receptor agonists DWH-146a and CGS21680 were not as effective in inhibiting the oxidative burst (53 and 9 nM, respectively; Fig. 2). DWH-146e inhibition of the stimulated neutrophil oxidative burst was antagonized by the selective A_{2A} AR antagonist ZM241385.

30   As shown in Fig. 3, JMR193 (1 nM) with rolipram (100 nM) synergistically decreased stimulated release of reactive oxygen species. Human neutrophils were primed with tumor necrosis factor-alpha (1 U/ml) and stimulated with f-met-leu-phe (1 µM). The vertical axis gives the percent

28

inhibition of oxidative activity as measured by luminol-enhanced chemiluminescence. Means SEM (n = 4 separate experiments. *p < 0.05 synergy between JMR193 and rolipram compared to additive activity.

As shown in Fig. 4, the highly selective A_{2A} adenosine receptor

5 antagonist ZM241385 (100 nM) (ZM) counteracted human neutrophil oxidative activity inhibited by JMR193 (10 nM) as measured by luminol-enhanced chemiluminescence. Means SEM of 4 separate experiments. *p = .0004 ZM241385 counteracted JMR193 inhibited oxidative activity.

E. Human neutrophil [cAMP]; and neutrophil adherence to a biological surface

10   A 24 well tissue culture plate was coated with human fibrinogen (5 mg/ml in 1.5% sodium bicarbonate; 0.5 ml/well; Sigma Chemical) overnight at 37°C in 5% CO_2. Neutrophils (3-4 × 10^6/0.5 ml/sample) were incubated within a well of the coated plate for 45 minutes in 0.5 ml of HBSS containing 0.1% HSA and ADA (1 U/ml) in the presence and absence of recombinant human TNFα (10

15 U/ml), DWH-146e (3-300 nM), rolipram (300 nM), and/or ZM241385 (100 nM). Following incubation, 0.5 ml HCl (0.2 N) was added to the wells and incubated for 45 minutes more at room temperature to extract the cAMP. The samples were then centrifuged in a microfuge for 2 minutes to remove cell debris. Half ml samples were frozen for cAMP analyses (B. Brooker et al., Science, 194, 270

20 (1976)). The wells were washed twice with normal saline and the remaining monolayer digested with 0.2 ml of 0.2 N NaOH containing SDS for 2 hours at room temperature. The protein samples were then frozen (-70°C) for later protein analysis to determine relative PMN adherence (K. P. Stowell et al., Anal. Biochem., 85, 572 (1978)).

25 Results

DWH-146e (30-300 nM) alone and synergistically with rolipram (300 nM) increased human neutrophil cAMP content and with rolipram synergistically decreased neutrophil adherence to a fibrinogen-coated surface (Fig. 5). The effects of DWH-146e (300 nM) + rolipram (300 nM) on neutrophil

30 cAMP production and adherence were counteracted by the selective A_{2A} adenosine receptor antagonist, ZM241385 (ZM; 100 nM). Mean SEM of 5 separate experiments. *p < 0.05 increased neutrophil [cAMP] compared to

29

without DWH-146e; **p < 0.05 decreased neutrophil adherence compared to no DWH-146e.

E.   Adherent human neutrophil oxidative activity

Methods. Using methods modified from Section E, neutrophils (2 ×

5   10^6/ml) from Ficoll-Hypaque separation were incubated 15 minutes 37°C in 0.45 ml of Hanks balanced salt solution containing 0.1% human serum albumin and adenosine deaminase (1U/ml), rolipram (300 nM), and DWH-146e (3-300 nM). Following incubation, cytochrome C (120 μM) and catalase (0.062 mg/ml) are added in the presence and absence of recombinant human tumor necrosis factor-

10   alpha (1U/ml) and 200 μl aliquots of cell suspension were immediately transferred to duplicate wells of a 96 well flat-bottomed tissue culture plate which had been coated overnight with human fibrinogen. The optical density of the samples were read at 550 nm against matched superoxide dismutase (200 U/ml) samples.

15   G. Results

As shown in Fig. 6, inhibition of tumor necrosis factor-alpha (TNF)-stimulated adherent human neutrophil superoxide release on a fibrinogen-coated surface was accomplished by rolipram (300 nM) and DWH-146e. DWH-146e decreased the oxidative burst of adhering neutrophils, and synergistically

20   decreased the oxidative burst in the presence of rolipram, which by itself did not affect neutrophil oxidative activity. The horizontal axis gives the DWH-146e concentration in nM and the vertical axis gives the amount of superoxide released by the neutrophils as measured by cytochrome c reduction. There was marked synergy with DWH-146e and the type IV PDE inhibitor, rolipram, to

25   decrease tumor necrosis factor-alpha-stimulated adherent human neutrophil oxidative activity. Means SEM of replicates from 4-5 separate experiments. *p < 0.05 decreased superoxide release compared to without DWH-146e; **p < 0.05 decreased superoxide release compared to with rolipram and without DWH-146e.

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EXAMPLE 18

Treatment of Ischemia/Reperfusion (I/R) Injury in Kidney with DWH-146e

To determine whether or not DWH-146e induced A_{2A} adenosine receptor activation reduces plasma creatinine at 24 and 48 hours following I/R injury in
5   rats, rat kidneys were subjected to 45 minutes ischemia and 24 or 48 hours of reperfusion. DWH-146e (0.004 μg/kg/min) or vehicle was administered continuously via minipump beginning 5 hours prior to I/R. As shown in Fig. 7, DWH-146e significantly decreased plasma creatinine in 7/7 rats (P < 0.05) and in 6/6 rats treated with DWH-146e (P < 0.001), at 24 and 48 hours, respectively.
10   To determine whether or not the effect of DWH-146e on reduction of plasma creatinine in rats subjected to I/R is A_{2A}-receptor mediated, rat kidneys were subjected to 45 minutes ischemia followed by 48 hours reperfusion. DWH-146e (0.004 μg/kg/min) was administered continuously via minipump beginning 5 hours prior ischemia. As shown in Fig. 8, the improvement in renal function
15   was reversed by the A_{2A} antagonist ZM-241385 (0.003 μg/kg/min-equimolar delivery rate compared with DWH-146e) (*P < 0.001 for Vehicle vs. DWH; **P < 0.05 DWH vs. DWH/ZM. N = 5 for Vehicle, DW; N = 6 for DWH/ZM. ANOVA followed by Bonferroni correction).

DWH-146e, at concentrations that have no hemodynamic effects,
20   prevents renal edema, necrosis and red cell pooling in the inner medulla.

The protection against renal damage afforded by DWH-146e (0.01 μg/kg/min s.c. for 48 hours) was correlated with a dramatic inhibition of neutrophil adherence to vascular endothelium. It is believed that inhibition by DWH-146e of the interaction between neutrophils and vascular endothelium is
25   responsible, at least in part, for the protection against renal damage.

To determine whether or not A_{2A}-AR activation reduces neutrophils in the outer medulla of rats subjected to I/R, using Neurolucida®, the kidney was viewed under 100 × mag and the entire kidney was drawn. PMNs were counted by viewing kidney sections under 250 × mag. Kidney sections were overlaid
30   with optical frames viewed under the microscope and all PMNs were counted within each frame. This system prevents counting of PMNs more than once. As shown in Fig. 9, the density of neutrophils was $15.65/mm^2$ for vehicle and $3.02/mm^2$ for DWH-146e treatment.

31

Example 19. Effect of DWH-146e on Lung Reperfusion Injury.

A. Methods. An isolated, whole blood-perfused, ventilated rabbit lung model was used. Donor rabbits underwent lung harvest after pulmonary arterial PGE_1 injection and Euro-Collins preservation solution flush, and lungs were preserved

5   for 18 hours at 4°C. Group I lungs (n=9) served as control subjects. Group II lungs (n=9) were reperfused with whole blood that was first passed through a leukocyte-depleting filter. In group III (n=9), DWH-146e was added to the blood reperfusate (25μg/kg) immediately before reperfusion and was administered throughout the reperfusion period (1μg/kg/min). All lungs were

10   reperfused for 30 minutes, and pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR), airway compliance (CPL) and arterial oxygenation were recorded. Mycloperoxidase activity (MPO) was recorded to quantify neutrophil sequestration, and wet/dry weight ratios were measured to demonstrate pulmonary edema.

15   B. Results. Arterial oxygentation in group II and group III was significantly higher than that of group I after 30 minutes of reperfusion (514.27 ± 35.80 and 461.12 ± 43.77 vs. 91.41 ± 20.58 mm Hg, $p<0.001$. As shown in Fig. 10, group III lungs displayed a progressive involvement in pO_2 throughout reperfusion. Leukocyte depletion in group II lungs improved arterial oxygenation in early

20   reperfusion. *p=0.004 (group II versus groups I and III); **p<0.001 (groups II and III versus group I).

As shown in Fig. 11, mean PVR in group II was significantly reduced when compared to controlled lungs (*p<0.001). PVR of group III lungs was significantly lower than even those lungs that underwent reperfusion with

25   leukocyte-depleted blood (**p<0.001 versus groups I and II). Pulmonary vascular resistance was significantly reduced in group III (22,783 ± 357 dynes·s·cm^{-5}) compared to both group II and group I (31,057 ± 1743 and 36,911 ± 2173 dynes·s·cm^{-5}, p<0.001). Airway compliance was improved in groups II and III when compared to group I (1.68 ± 0.08 and 1.68 ± 0.05 vs. 1.36 ± 0.13,

30   p=0.03). Microvascular permeability in group III was reduced to 106.82 ± 17.09 compared with 165.70 ± 21.83 ng Evans-blue dye/gm tissue in group I (p=0.05). As shown in Fig 12, myeloperoxidase activity in group III was significantly lower than in group I (*p=0.03). MPO=myeloperoxidase. Group III

32

myeloperoxidase activity was 39.88 ± 4.87 compared with 88.70 ± 18.69 ΔOD/gm/min in group I (p=0.03), and group II myeloperoxidase activity was 56.06 ± 7.46.

C. Conclusions. DWH-146e reduced lung neutrophil sequestration and
5   dramatically improved pulmonary graft function. Neutrophils are important components of the inflammatory cascade of reperfusion injury and their source may include both the circulating blood and the lung graft itself. Selective adenosine-A_{2A} activation interrupts the neutrophil-mediated inflammatory response and reduces lung reperfusion injury following transplantation.

10   Under light microscopy, control lungs in group I showed severe leukocyte infiltration and edema formation in the alveolar spaces after 18 hours of ischemic storage and 30 minutes of reperfusion. In group II, lungs that underwent reperfusion with leukocyte-depleted blood and in group III lungs (that received DWH-146e during reperfusion, this infiltration was much less.

15

Example 20. Effect of DWH-146e on Neointimal Formation after Arterial Injury. Leukocyte activation with release of inflammatory cytokines occurs after percutaneous coronary intervention and may play a role in restenosis. In the mouse, robust neointima formation in the presence of an intact endothelial
20   lining occurs after ligation of the common carotid artery. Using this model, C57/BL6 mice were randomized at the time of carotid ligation to a 7 day infusion via osmotic pump of DWH-146e, (n=7), or vehicle (n=8).

At 14 days after carotid ligation, histomorphometry demonstrated a significant reduction in neointimal area (0.005 ± 0.004 mm^2 vs. 0.021 ± 0.014
25   mm^2, p=0.02) and neointimal to medial area ratio (0.13 ± 0.07 vs. 0.64 ± 0.44, p=0.01) in the treated animals compared to controls. Medial area was similar in the two groups (0.034 ± 0.007 mm^2 vs. 0.036 ± 0.009 mm^2, p=0.81). This benefit in limiting neointimal growth persisted to 28 days. Figure 13 summarizes the effect of DWH-146e to inhibit neointimal growth in the mouse
30   LCCA model. These experiments demonstrate that, in a mouse carotid artery ligation model, prolonged A_{2A} stimulation (7 days) by DWH-146e results in a significant reduction in neointimal formation for at least 21 days, possibly through its effect on leukocyte activation and function.

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Example 21. Inhibition of Endotoxin-stimulated Human Monocyte TNFα Release.

A. Materials.

Ficoll-hypaque was purchased from ICN (Aurora, OH) and Cardinal
5 Scientific (Santa Fe, NM) and Accurate Chemicals and Scientific (Westbury,
NY). Endotoxin (lipopolysaccharide; *E. coli* 0111B4) was from List Biologicals
(Campbell, CA). Hanks balanced salt solution (HBSS), and limulus amebocyte
lysate assay kit were from BioWittaker (Walkersville, MD). Human serum
albumin (HSA) was from Cutter Biological (Elkhart, IN). ZM241385 (4-(2-[7-
10 amino-2-(2-furyl)[1,2,4]-triazolo[2,3-a][1,3,5]triazin-5-yl amino]ethyl)phenol)
was a gift from Simon Poucher, Zeneca Pharmaceuticals, Cheshire, UK. Stock
solutions (1 mM and 10 mM in DMSO) were made and stored at -20°C.

B. Production of TNFα by purified human adherent monocytes.

Methods: A monocyte rich monolayer (>65% monocytes) was prepared by
15 incubating 1 ml of the mononuclear leukocyte fraction (5 × 10^5/ml) from a
Ficoll-Hypaque separation (A. Ferrante et al., J. Immunol. Meth., 36, 109
(1980)) in wells of a 24 well tissue culture plate (1 hr; 37°C; 5% CO_2). The non-
adherent leukocytes were removed by washing and culture medium (1 ml Hanks
balanced salt solution, containing 0.1% human serum albumin, adenosine
20 deaminase [5 U/ml] and 1% heat-inactivated autologous serum) added to the
wells containing the adherent mononuclear cells. As stated, the following were
added: (1) endotoxin (100 ng/ml) and the A_{2A} AR selective antagonist
ZM241385 (100 nM) and, (2) A_{2A} adenosine receptor selective agonists JMR193
(1-1000 nM), DWH146e (1-1000 nM) and CGS21680 (10-1000 nM). The
25 samples were then incubated for 4 hours (37°C; 5% CO_2) and the supernatants
harvested. Any suspended cells were removed by centrifugation and the cell-free
samples frozen (-70°C). TNFα was assayed in the cell-free supernatants (n=6)
by an ELISA kit (Coulter/Immunotech, Miami, FL).

C. Results.

30 As shown in Figure 10, 14, the A_{2A} adenosine receptor agonists decreased
endotoxin-stimulated adherent monocyte production of TNFα. The A_{2A} AR
selective antagonist ZM241385 (100 nM) antagonized the effects of JMR193 on
TNFα production (Figure 15).

Thus, DWH146e and JMR193 decrease LPS endotoxin-stimulated TNFα production by human monocytes by a mechanism that is dependent upon agonist binding to A_{2A} adenosine receptors.

5   **EXAMPLE 22. Activity of DWH-146e in Murine Peritonitis Model.**

Preliminary experiments with experimental peritonitis have involved the injection of zymosan (Zym) as a potent stimulus of inflammation (Y. Zhang et al., Eur. J. Pharmacol., 313, 237 (1996)). As shown in Figure 16, following injection of zymosan, the mean leukocyte concentration as determined in a
10   neubauer hemocytometer was $7,325 \pm 1,893/mm^3$. Intraperitoneal injection of DWH-146e at a dosage of 2.5 µg/kg one hour prior to zymosan inhibited the development of peritonitis with a mean ± SEM leukocyte concentration of 2,012 $\pm 374/mm^3$ 6 hours later ($p < 0.05$). Thus, these studies demonstrate that the A_{2A} AR is instrumental in mediating PMN traversal into the peritoneum following
15   zymosan challenge.

Example 23. Cardioprotection Mediated by the Anti-inflammatory Effect of JMR193

The compounds of the invention were tested by inducing myocardial
20   stunning, a form of cardiac injury that occurs following repetitive, transient periods of interrupted coronary blood flow, by repeated occlusion of the blood supply of a coronary artery.

A. Effect of four cycles of occlusion-reperfusion.

The left anterior descending (LAD) coronary artery of a group of dogs
25   was isolated and encircled with a snare occluder. The dogs LAD artery blood supply was occluded 4 times, for 5 minutes. Following each occlusion blood flow was restored for ten minutes. One group of six dogs were infused with a solution containing the acetate compound (JMR193), prepared in Example 15 (JMR193), (0.01 µg/kg/min) after each occlusion period. A second group of six
30   dogs were infused with a solution containing the vehicle (carrier). After the last occlusion-reperfusion cycle the animals cardiac function was monitored for 3 hours.

35

The results are illustrated in Figures 17 and 18. Figure 17 shows the systolic left ventricular (LV) thickening response in 6 control dogs. Cardiac thickening was reduced by approximately 50% 3 hours after the last occlusion. Figure 18 shows the LV thickening response in the 6 dogs that received an i.v.

5   infusion of the test compound, JMR193 (0.01 μg/kg/min), beginning during the baseline period and continuing throughout the experiment. The cardiac function, with JMR193 infusion, returned to nearly normal as early as 90 min post reperfusion.

10   B. Effect of ten cycles of occlusion-reperfusion.

Two additional groups of dogs were subjected to ten (rather than 4) occlusion-reperfusion cycles, where the each occlusion was 5 minutes, interspersed by 5 minutes of reperfusion. In this example, two of the animals were infused with a solution containing the acetate compound (JMR193),

15   prepared in Example 15, (0.01 μg/kg/min) after each occlusion period. Another three animals were infused with a solution containing the vehicle (carrier). After the last occlusion-reperfusion cycle the animals cardiac function was monitored for 3 hours.

The results are illustrated in Figures 19 and 20. Figure 19 shows the

20   systolic left ventricular thickening response in the 3 control dogs. This was a more severe cardiac insult, than in Example 23A, and as a result the LV thickening was completely absent early after reperfusion and remained akinetic for 3 hours. Figure 20 shows the LV thickening in the 2 dogs that received an i.v. infusion of the test compound, JMR193 (0.01 μg/kg/min) beginning during

25   the baseline period and continuing throughout the experiment. Compared with the control group, the dogs that received the JMR193 infusion showed a significant and marked improvement in cardiac function immediately after reperfusion which persisted for 3 hours.

C. Effect of acetate compound JMR193 on uptake of neutrophils during

30   occlusion-reperfusion.

Some animals were administered radiolabeled neutrophils. (Neutrophils were isolated from dog blood, incubated with a compound containing ^{99m}Tc, and reinjected into the dogs.) The ^{99m}Tc- labeled neutrophils were administered

36

intravenously as a marker to determine the level of inflammation in the reperfusion zone, following four ischemic-reperfusion cycles. The inflammation from the occlusion-reperfusion cycles caused adherence of the radioactive neutrophils and was quantified with a gamma-camera. The

5   neutrophil adherence was inhibited by the JMR193. The results are illustrated in Figure 21 where the localization of ^{99m}Tc-labeled neutrophils in the dogs treated with vehicle alone (solid bars) is greater than the JMR193 treated (striped bars) dogs. Thus, the reduction of radiolabeled neutrophils in the central ischemic zone caused by the JMR193 infusion illustrates the reduction of (*) cardiac

10   inflammation.

The studies described in Examples 23A and 23B indicate that cardiac inflammation plays a significant injurious role in causing myocardial stunning. In addition, the administration of an adenosine A_{2A} receptor agonist such as, for example, JMR-193 either prevents mild stunning (Figures 17 and 18) or

15   significantly attenuates myocardial dysfunction accompanying severe stunning (Figures 19 and 20).

All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The invention has been described with reference to various specific and preferred

20   embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

37

What is claimed is:

1.   A compound of the formula (I):

(I)

$$R^1-C\equiv C \quad [\text{purine ribonucleoside structure with } N(R)_2, X, O, OH, OH]$$

wherein (a) each R is individually hydrogen, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, phenyl or phenyl(C_1-C_3)-alkyl;

(b) X is -CH_2OH, -CO_2R^2, -$OC(O)R^2$ or $C(O)NR^3R^4$;

(c) each of R^2, R^3 and R^4 is individually H, C_{1-6}-alkyl; C_{1-6}-alkyl substituted with 1-3 C_{1-6}-alkoxy, C_3-C_7 cycloalkyl, C_{1-6}-alkylthio, halogen, hydroxy, amino, mono(C_{1-6}-alkyl)amino, di(C_{1-6}-alkyl)amino, or C_{6-10}-aryl, wherein aryl may be substituted with 1-3 halogen, C_{1-6}-alkyl, hydroxy, amino, mono(C_{1-6}-alkyl)amino, or di(C_{1-6}-alkyl)amino; C_{6-10}-aryl; or C_{6-10}-aryl substituted with 1-3 halogen, hydroxy, amino, mono(C_{1-6}-alkyl)amino, di(C_{1-6}-alkyl)amino or C_{1-6}-alkyl;

(d) R^1 is (X-(Z)-)$_n$[(C_3-C_{10})cycloalkyl]-(Z')- wherein Z and Z' are individually (C_1-C_6)alkyl, optionally interrupted by 1-3 S or noperoxide O, or is absent, and n is 1-3; or a pharmaceutically acceptable salt thereof.

2.   A compound of claim 1 wherein X is -CH_2OH or -$C(O)NR^3R^4$.

3.   A compound of claim 2 wherein R^3 is H and R^4 is (C_1-C_4)alkyl.

4.   A compound of claim 1 wherein each R is H or (C_1-C_4)alkyl.

5.   A compound of claim 1 wherein Z' is -CH_2- or -CH_2-CH_2-.

38

6.   A compound of claim 5 wherein Z is -CH$_2$- or -CH$_2$-CH$_2$-.

7.   A compound of claim 1 wherein R^1 comprises cyclohexyl or cyclopentyl.

8.   A compound of claim 7 wherein X is (C$_1$-C$_4$)alkoxycarbonyl, C(O)NR^{3}R^4 or acetoxymethyl.

9.   A compound of claim 7 wherein X is carboxy.

10.   A compound of claim 7 wherein X-Z and Z' are *trans*.

11.   A compound of claim 1 wherein R is H, X is ethylaminocarbonyl, and R^1 is 2-(4-methoxycarbonyl-cyclohexylmethyl)ethynyl or 2-(4-carboxy-cyclohexylmethyl)ethynyl.

12.   A compound of claim 1 wherein R is H, X is ethylaminocarbonyl, and R^1 is 2-(4-acetoxymethyl-cyclohexylmethyl)ethynyl.

13.   [4-(3-{9-(4S,5S,2R,3R)-5-(N-Ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl}prop-2-ynyl)cyclohexyl]methyl acetate.

14.   [(2S,3S,4R,5R)-5-(6-amino-2-{3-[4-(hydroxymethyl)cyclohexyl]prop-2-ynyl}purin-9-yl)-3,4-dihydroxyoxolan-2-yl]-*N*-ethylcarboxamide.

15.   Methyl-4-(3-{9-[(4S,5S,2R,3R)-5-(N-ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl)}prop-2-ynyl)cyclohexane-carboxylate.

16.   4-(3-{9-[(4S,5S,2R,3R)-5-(N-Ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl)}prop-2-ynyl)cyclohexane carboxylic acid.

17.   A compound of formula (I):

39

(I)

$$R^1—C\equiv C$$

N(R)$_2$ structure with purine ring, X, O, OH OH

wherein (a) each R is individually hydrogen, C_1-C_6 alkyl, C_3-C_7 cycloalkyl, phenyl or phenyl(C_1-C_3)-alkyl;

(b) X is -CH$_2$OH, -CO$_2$R^2, -OC(O)R^2 or C(O)NR^{3}R^4;

(c) each of R^2, R^3 and R^4 is individually H, C_{1-6}-alkyl; C_{1-6}-alkyl substituted with 1-3 C_{1-6}-alkoxy, C_3-C_7-cycloalkyl, C_{1-6}-alkylthio, halogen, hydroxy, amino, mono(C_{1-6}-alkyl)amino, di(C_{1-6}-alkyl)amino, or C_{6-10}-aryl, wherein aryl may be substituted with 1-3 halogen, C_{1-6}-alkyl, hydroxy, amino, mono(C_{1-6}-alkyl)amino, or di(C_{1-6}-alkyl)amino, C_{6-10}-aryl, or C_{6-10}-aryl substituted with 1-3 halogen, hydroxy, amino, mono(C_{1-6}-alkyl)amino, di(C_{1-6}-alkyl)amino or C_{1-6}-alkyl;

(d) each of R^3 and R^4 is individually hydrogen, C_{3-7}-cycloalkyl, or any of the meanings of R^2; and

(e) R^1 is (X-(Z)-)$_n$[(C$_3$-C$_{10}$)cycloalkyl]-(Z')- wherein Z and Z' are individually (C$_1$-C$_6$)alkyl, optionally interrupted by 1-3 S or non-peroxide O, or is absent, and n is 1-3; or a pharmaceutically acceptable salt thereof, for use in medical therapy.

18.   A compound of any of claims 1-16 wherein the medical therapy is inhibition of an inflammatory response.

19.   The compound of claim 17 wherein X is -CH$_2$OH or -C(O)NR^{3}R^4.

20.   The compound of claim 19 wherein R^3 is H and R^4 is (C$_1$-C$_4$)alkyl.

21.   The compound of claim 17 wherein each R is H or (C$_1$-C$_4$)alkyl.

40

22.   The compound of claim 17 wherein Z' is -CH$_2$- or -CH$_2$-CH$_2$-.

23.   The compound of claim 17 wherein Z is -CH$_2$- or -CH$_2$-CH$_2$-.

5   24.   The compound of claim 17 wherein R^1 comprises cyclohexyl or
cyclopentyl.

25.   The compound of claim 24 wherein X is (C$_1$-C$_4$)alkoxycarbonyl or
acetoxymethyl.
10

26.   The compound of claim 24 wherein X is carboxy.

27.   The compound of claim 24 wherein X-Z and Z' are *trans*.

15 28.   The compound of claim 17 wherein R is H, X is ethylaminocarbonyl and
R' (R1) is 2-(4-methoxycarbonyl-cyclohexylmethyl)ethynyl or 2-(4-carboxy-
cyclohexylmethyl)ethynyl.

29.   The compound of claim 17 wherein R is H, X is ethylaminocarbonyl, and
20 R^2 (R1) is 2-(4-acetoxymethyl-cyclohexylmethyl)ethynyl.

30.   The compound of claim 17 which is [4-(3-{9-(4S,5S,2R,3R)-5-(N-
ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl}prop-2-
ynyl)cyclohexyl]methyl acetate.
25

31.   The compound of claim 17 which is [(2S,3S,4R,5R)-5-(6-amino-2-{3-[4-
(hydroxymethyl)cyclohexyl]prop-2-ynyl}purin-9-yl)-3,4-dihydroxyoxolan-2-yl]-
N-ethylcarboxamide.

30 32.   The compound of claim 17 which is methyl-4-(3-{9-[(4S,5S,2R,3R)-5-
(N-ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl)}prop-2-
ynyl)cyclohexane-carboxylate.

41

33.   The compound of claim 17 which is 4-(3-{9-[(4S,5S,2R,3R)-5-(N-ethylcarbamoyl)-3,4-dihydroxyoxolan-2-yl]-6-aminopurin-2-yl)}prop-2-ynyl)cyclohexane carboxylic acid.

5   34.   The compound of claim 17 wherein the medical therapy further comprises the use of a Type IV phosphodiesterase inhibitor.

35.   The compound of claim 17 wherein the inhibitor is rolipram.

10   36.   The compound of claim 18 wherein the inflammatory response is due to ischemia.

37.   The compound of claim 18 wherein the inflammatory response is due to atherosclerosis.

15

38.   The compound of claim 18 wherein the inflammatory response is due to an autoimmune disease.

39.   The compound of claim 18 wherein the inflammatory response is due to
20   ischemia/reperfusion injury.

40.   The compound of claims 18 wherein the inflammatory response is at the heart, kidney, or lung.

25   41.   The compound of claim 18 wherein the inflammatory response is due to stroke, traumatic brain injury, or spinal cord injury.

42.   The compound of claim 18 wherein the inflammatory response is due to organ, tissue or cell transplantation.

30

43.   The compound of claim 18 wherein the inflammatory response is due to infection.

44.   The compound of claim 18 wherein the inflammatory response is due to a skin disease.

45.   The compound of claim 18 wherein the inflammatory response is due to angioplasty, stent placement, shunt placement or grafting.

46.   The compound of claim 18 wherein the inflammatory response is due to an allergic disease.

47.   The compound of claim 18 wherein the inflammatory response is due to a wasting disease.

48.   The compound of claim 18 wherein the inflammatory response is due to immunosuppressive therapy.

49.   Use of a compound of claims 1-16 to prepare a medicament useful for treating an inflammatory response.

50.   The use of claim 49 wherein the medicament comprises a Type IV phosphodiesterase inhibitor.

51.   The use of claim 50 wherein the phosphodiesterase inhibitor is rolipram.

52.   The use of claim 50 wherein the medicament comprises a liquid carrier.

53.   The use of claim 50 wherein the medicament is adapted for parenteral administration.

FIG. 1

	JMR193	DWH146e	CGS21680	ACID DWH146
EC50	0.8140	0.3164	9.214	52.92
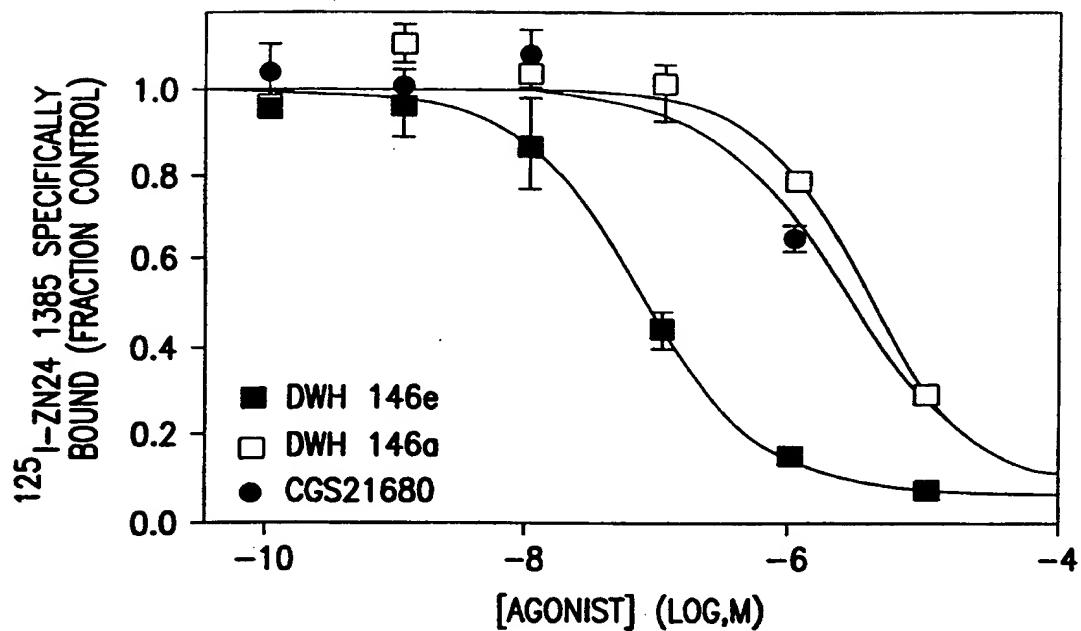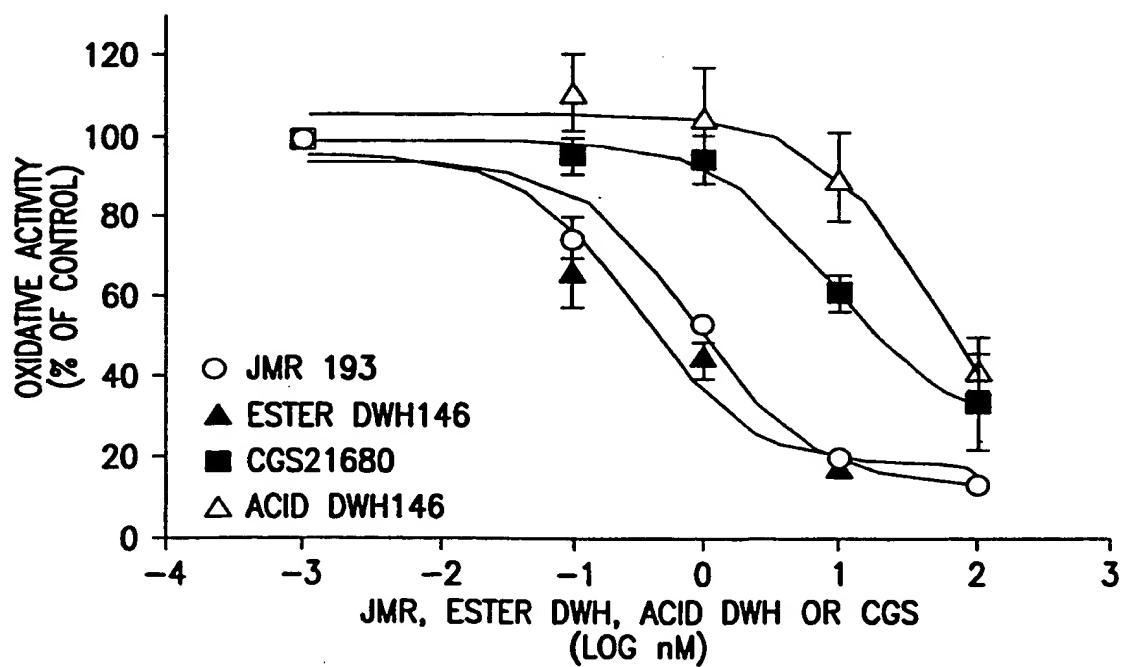
FIG. 2
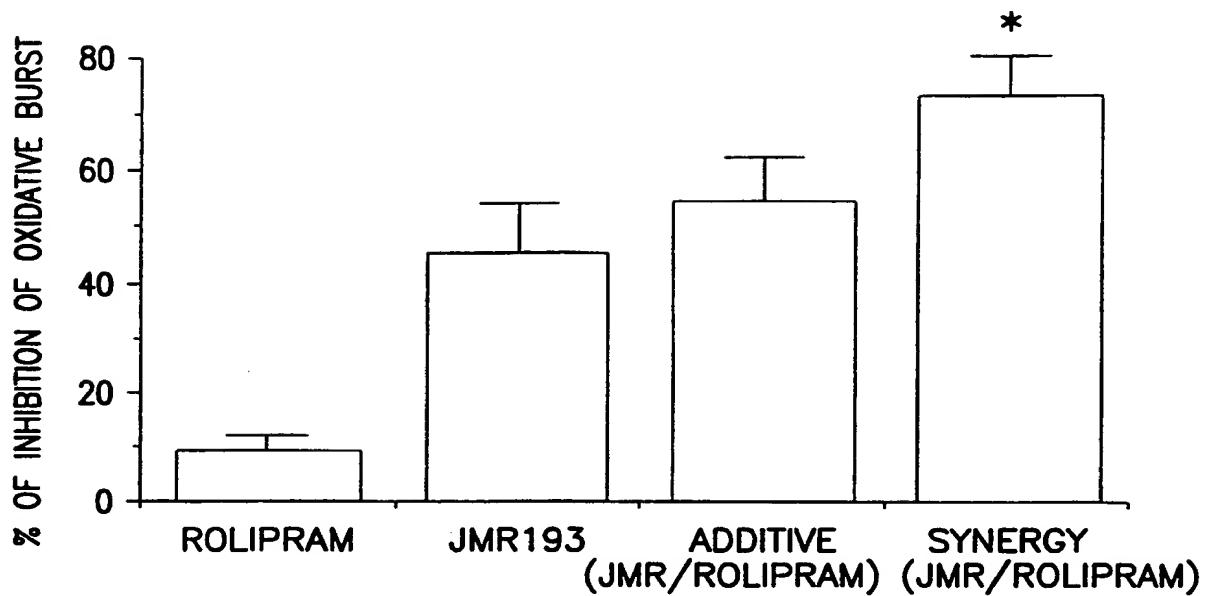
FIG. 3
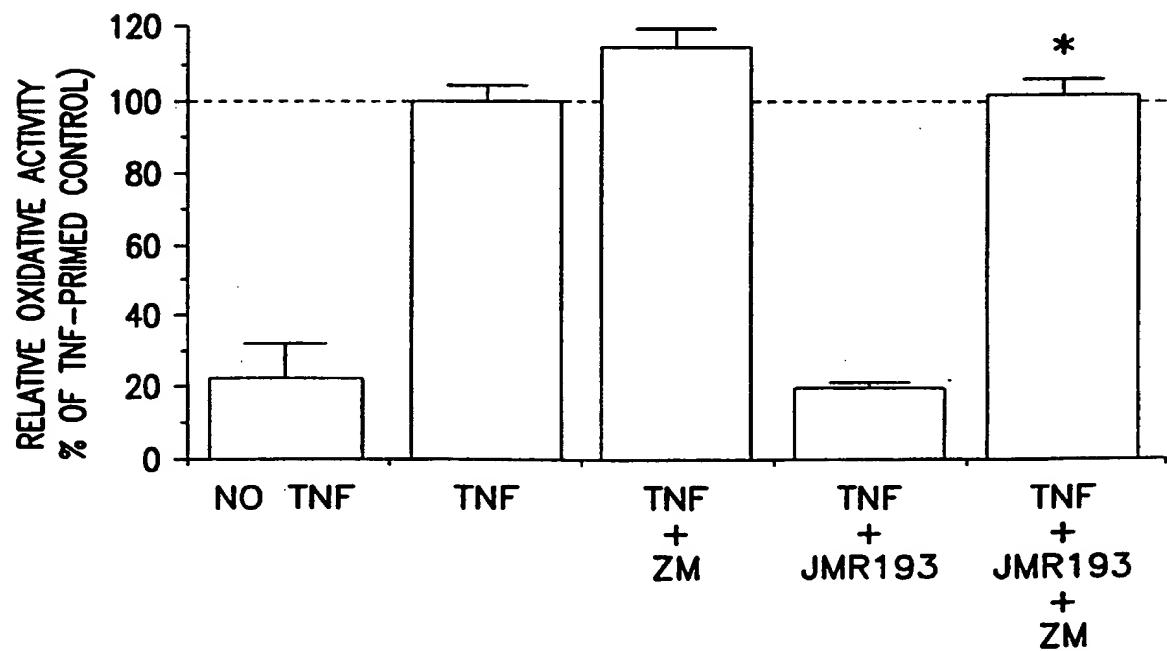
FIG. 4

DWH146e (LOT 1) ESPECIALLY WITH ROLIPRAM (300nM) INCREASE PMN cAMP AND DECREASES TNF-STIMULATED PMN ADHERENCE TO A FIBRINOGEN COATED SURFACE (MEAN ± SEM) n=5 EXPERIMENTS
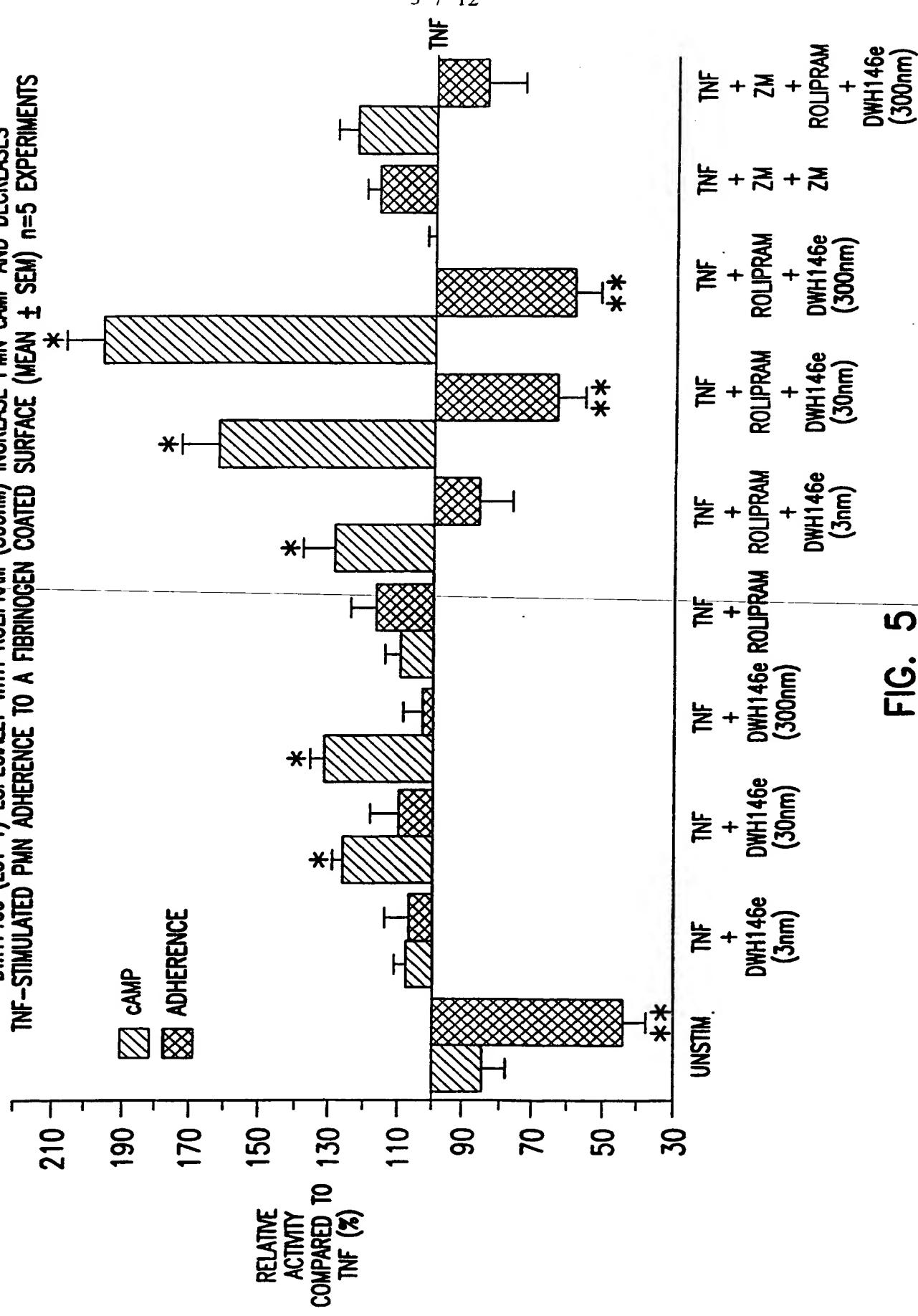
FIG. 5

INHIBITION OF TNF-STIMULATED ADHERENT PMN SUPEROXIDE RELEASE ON A FOBRINOGEN
COATED SURFACE ± ROLIPRAM (300nM) BY DWH146e
DOSE RESPONSE MEAN ± SEM 4-5 DONORS

NO ROLIPRAM

+ROLIPRAM
UNSTIMULATED
BASELINE

DWH146e (nM)

SUPEROXIDE
(NMOLES IN 150MIN)

FIG. 6

FIG. 7

FIG. 8

VEHICLE   DWH−146

FIG. 9

—O—CONTROL (I) —□—FILTER (II) —▲—A2A (III)

FIG. 10

FIG. 11

FIG. 12

○ DWH−146e   ● CONTROL

FIG. 13

(ALL SAMPLES WITH ADA 5U/ml)

A_{2A} ADENOSINE AGONIST (LOG M)

	JMR193	DWH146e	CGS21680
EC50	6.320e−009	3.457e−008	5.689e−006
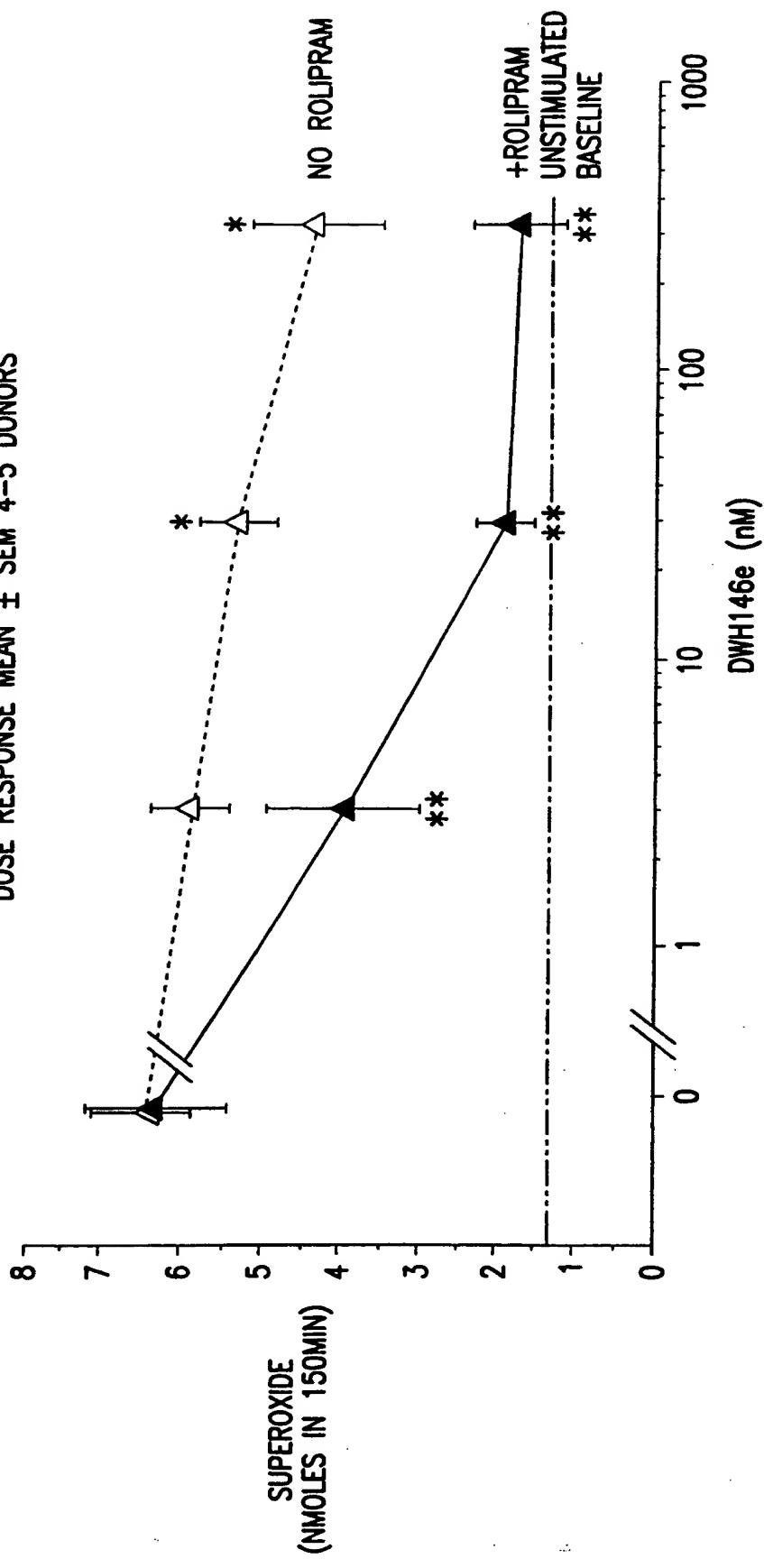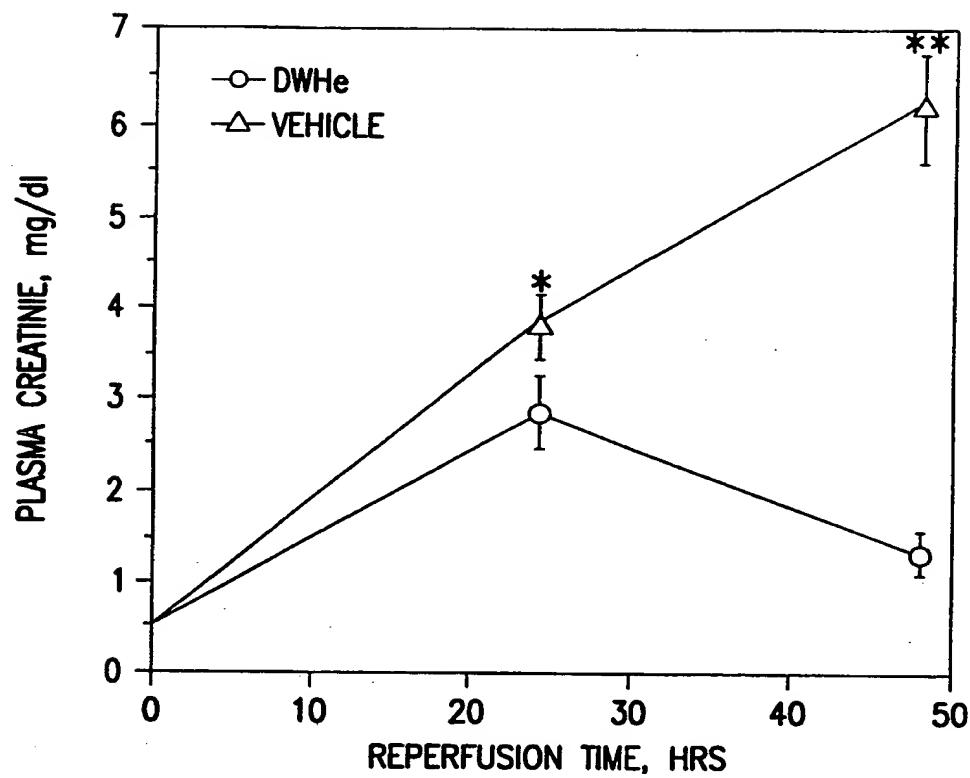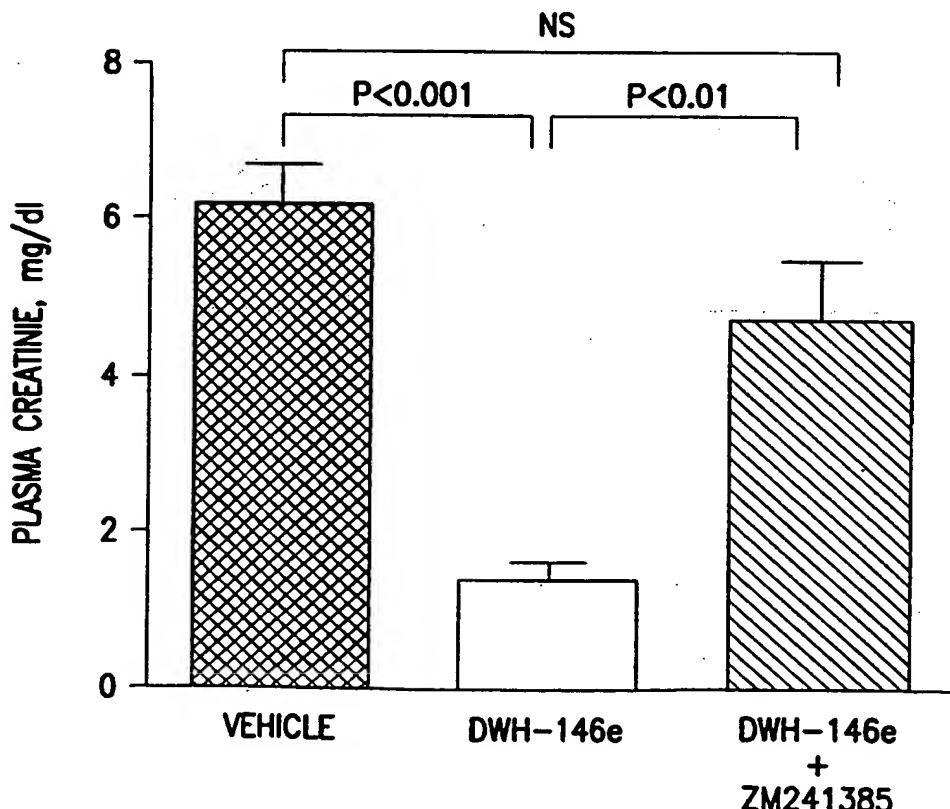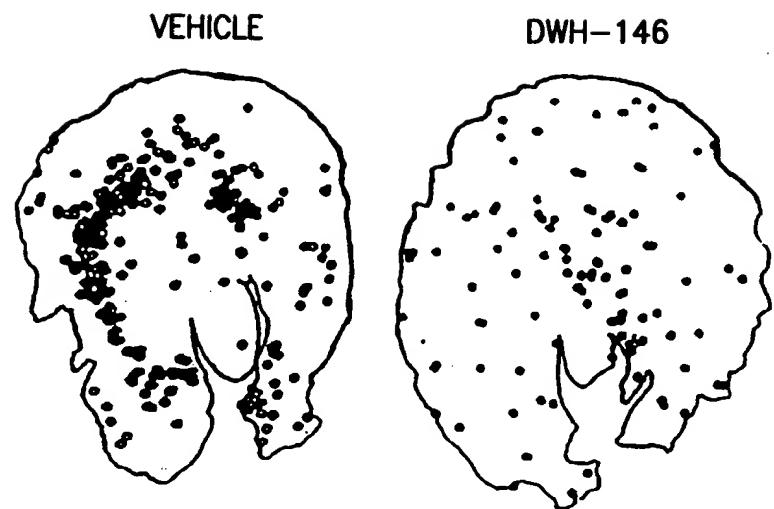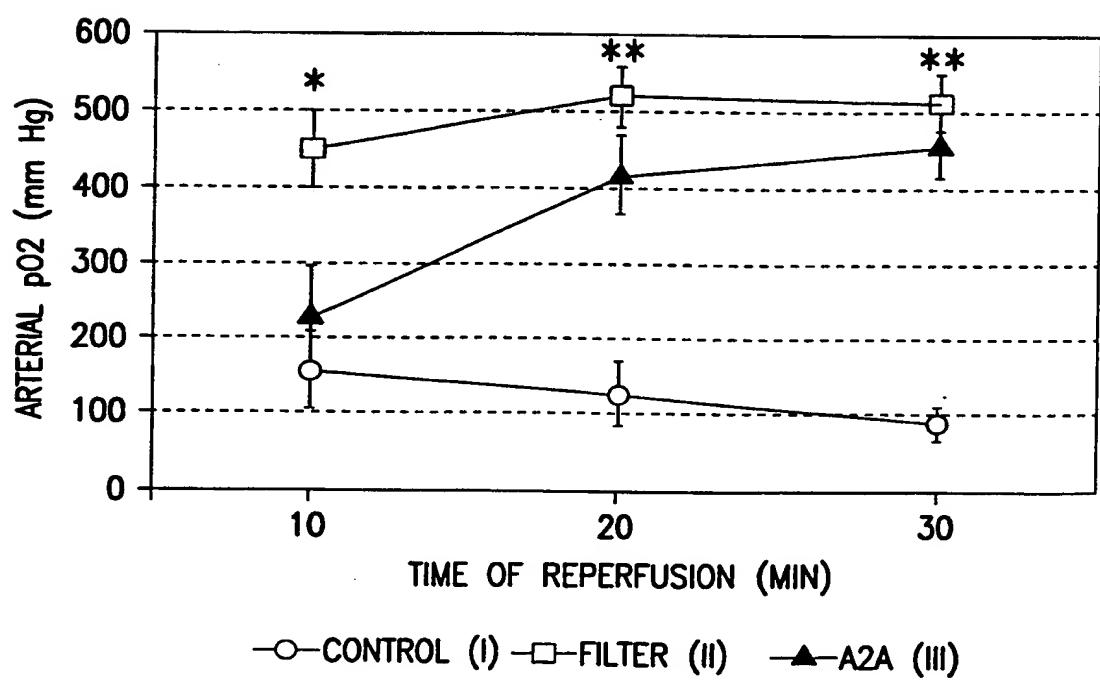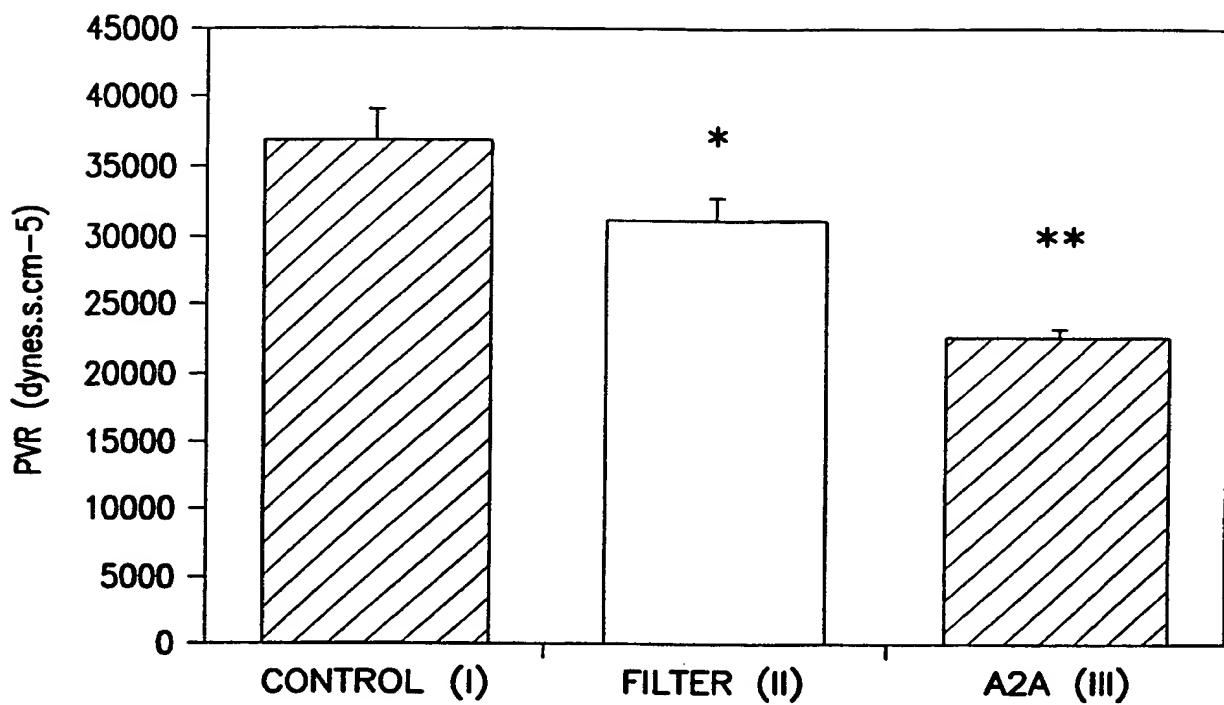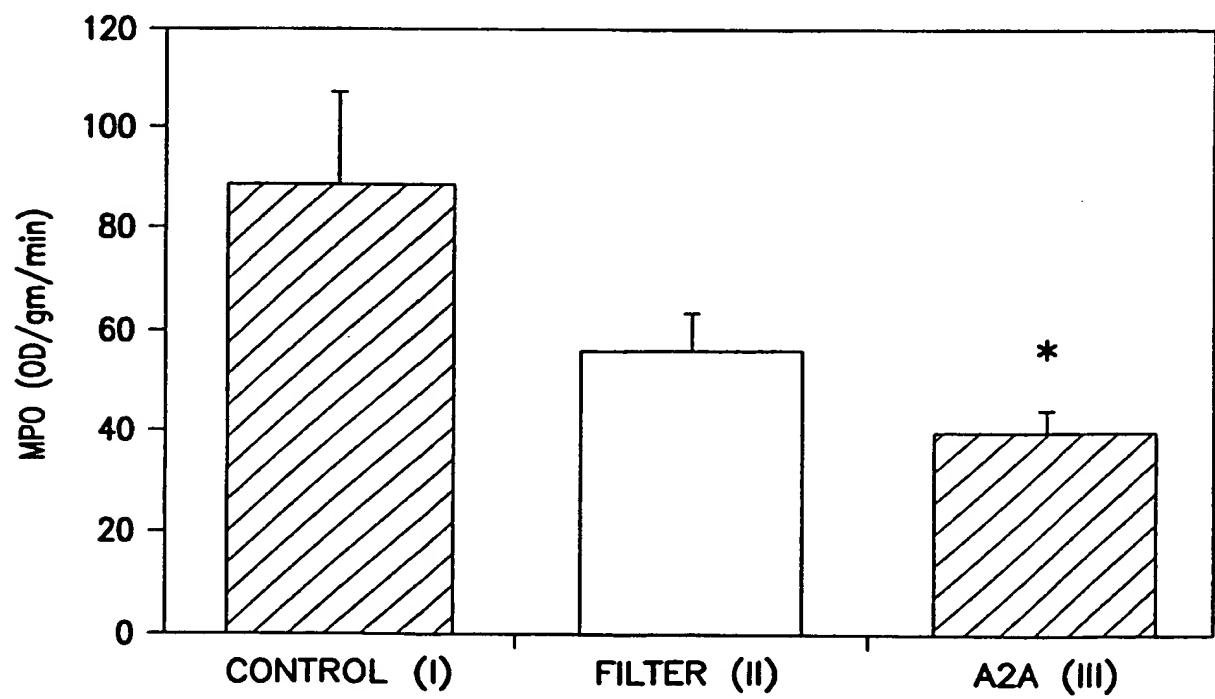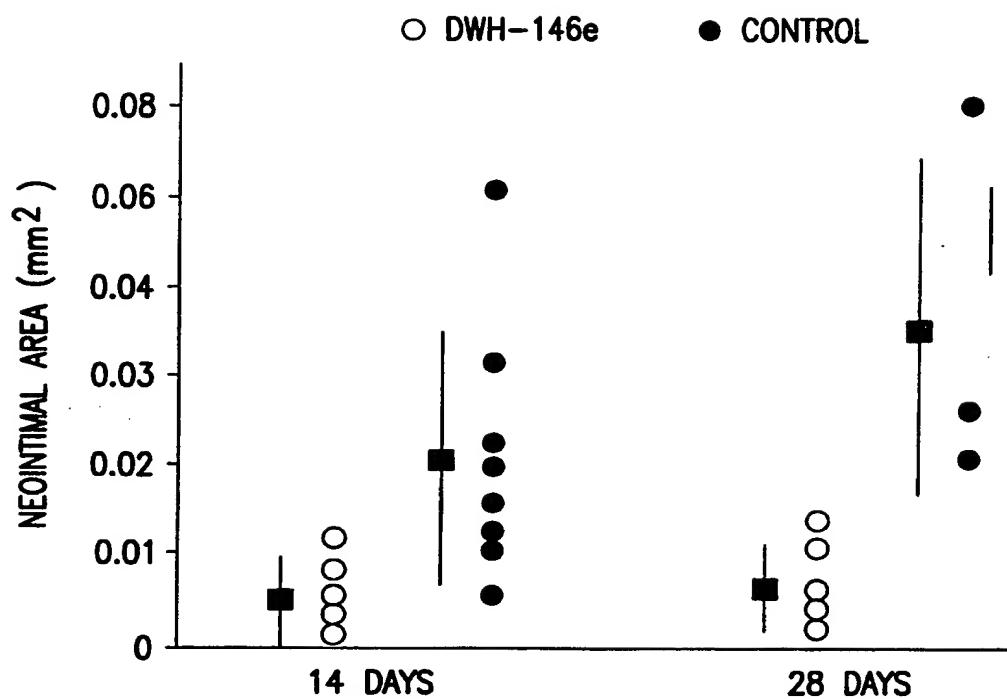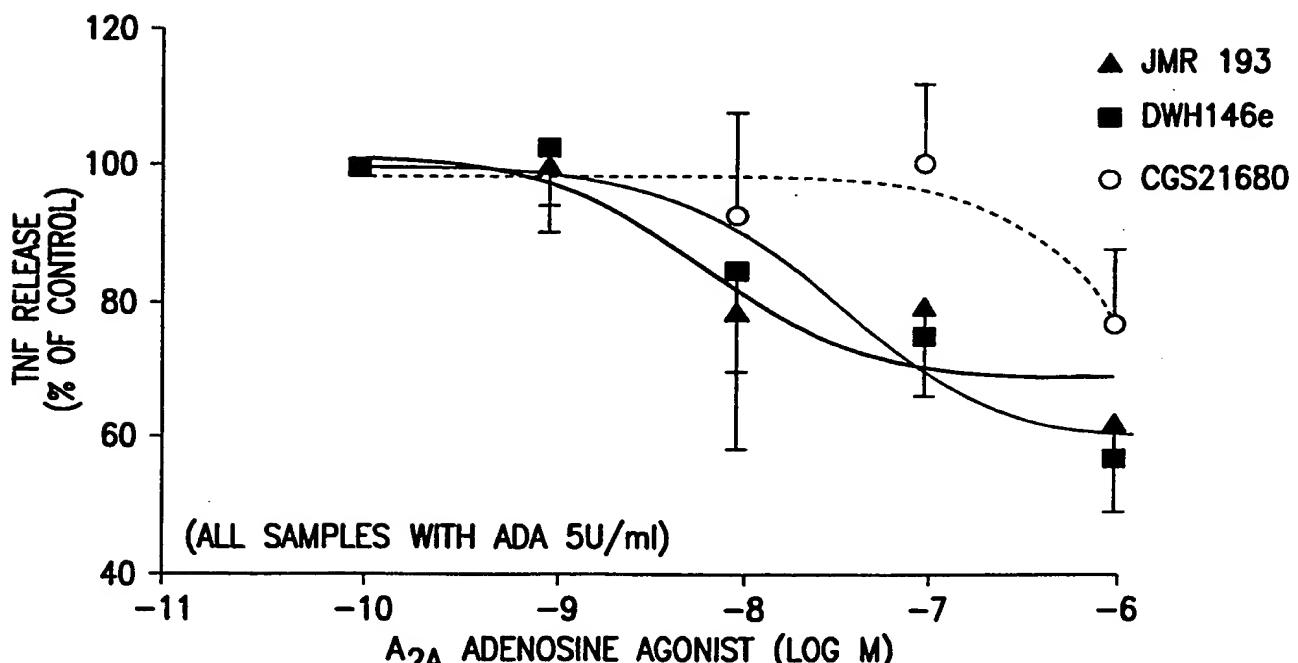
FIG. 14
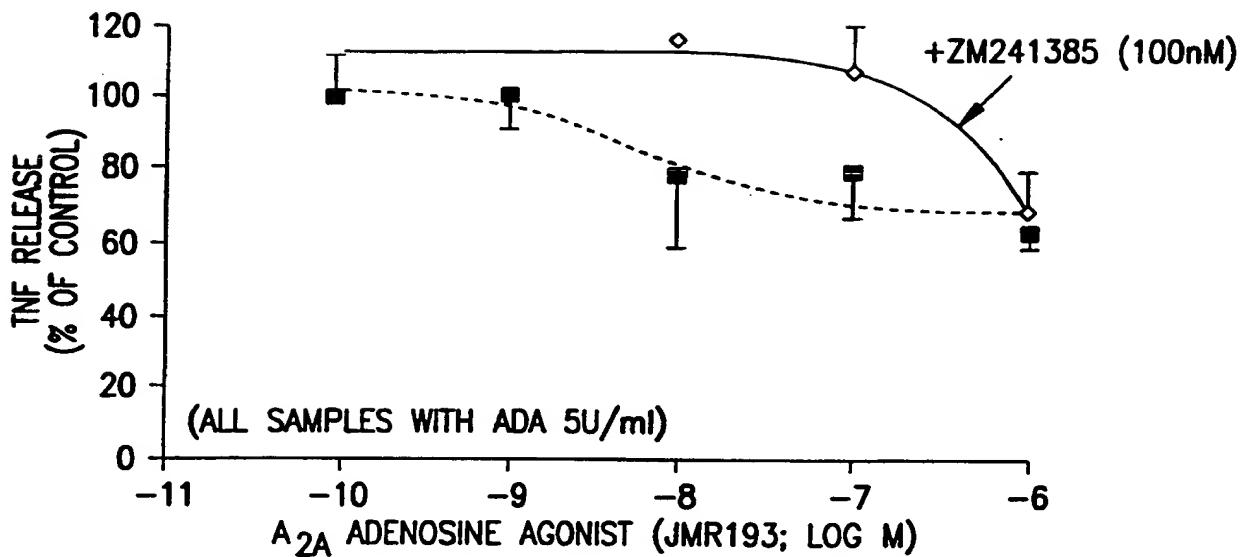
FIG. 15

FIG. 16

FIG. 17

FIG. 18

CONTROL

FIG. 19

TEST COMPOUND
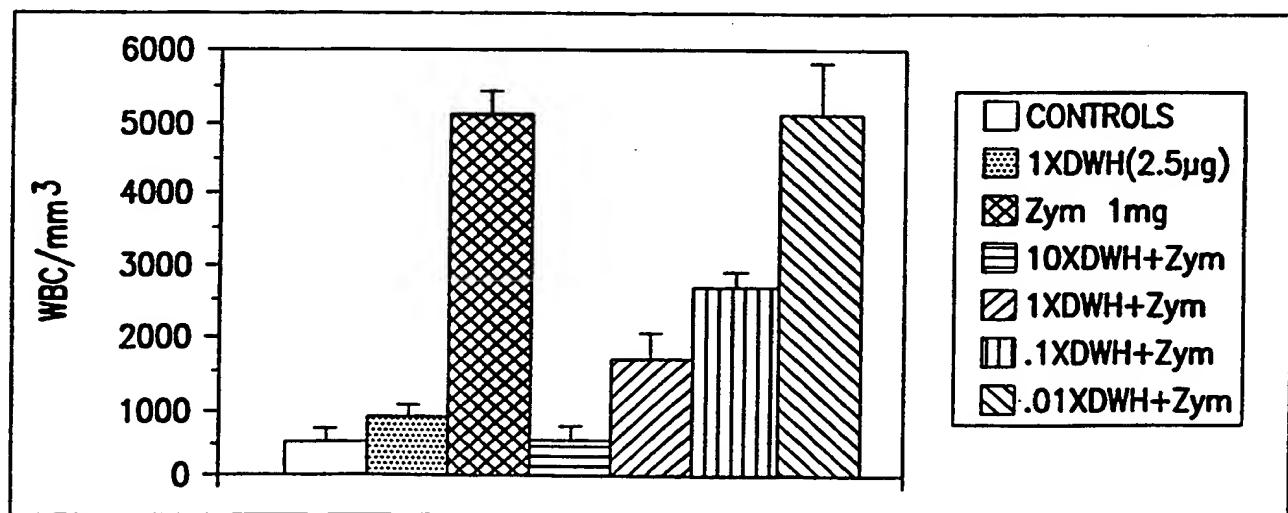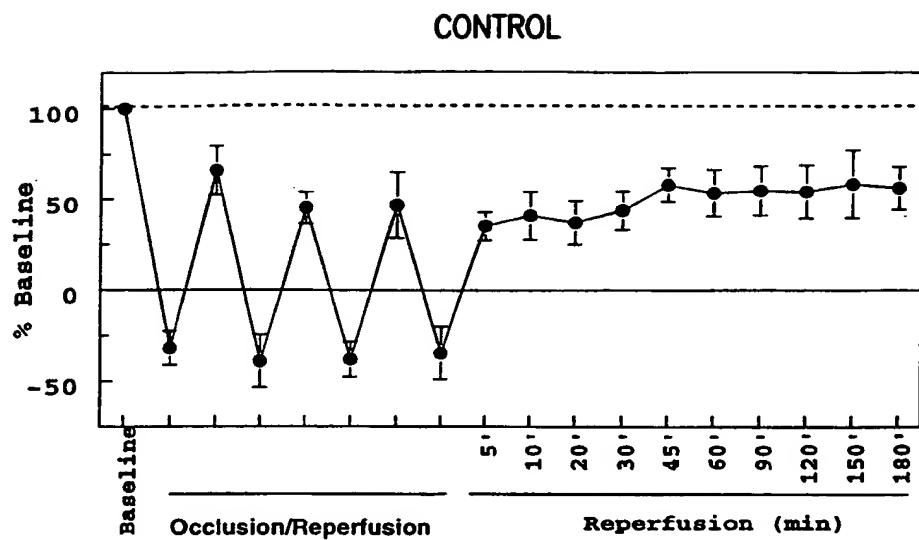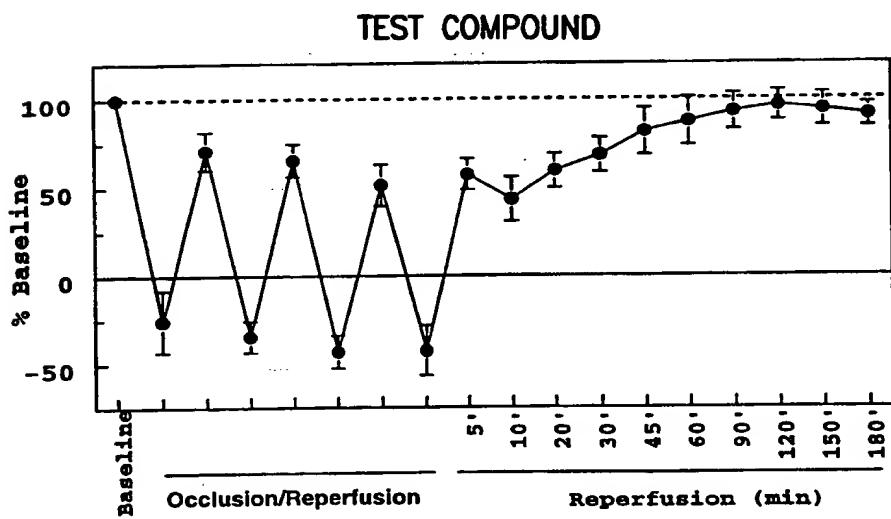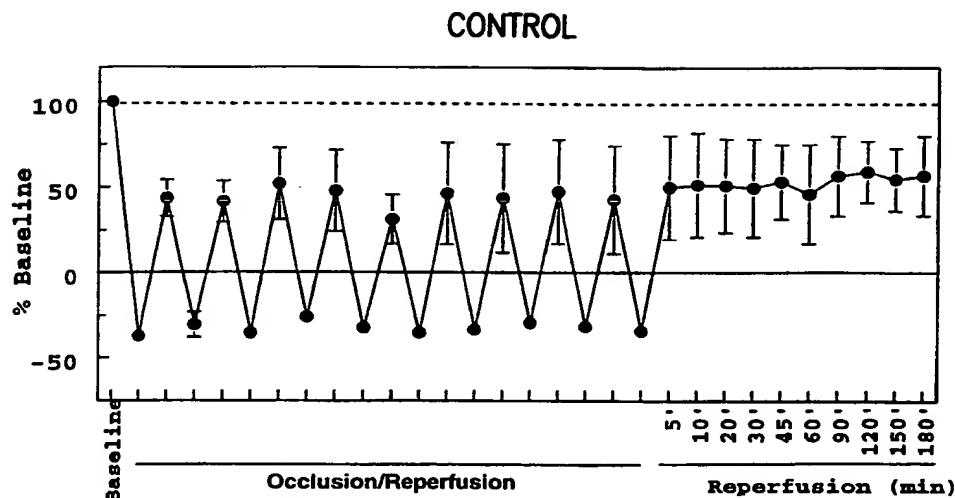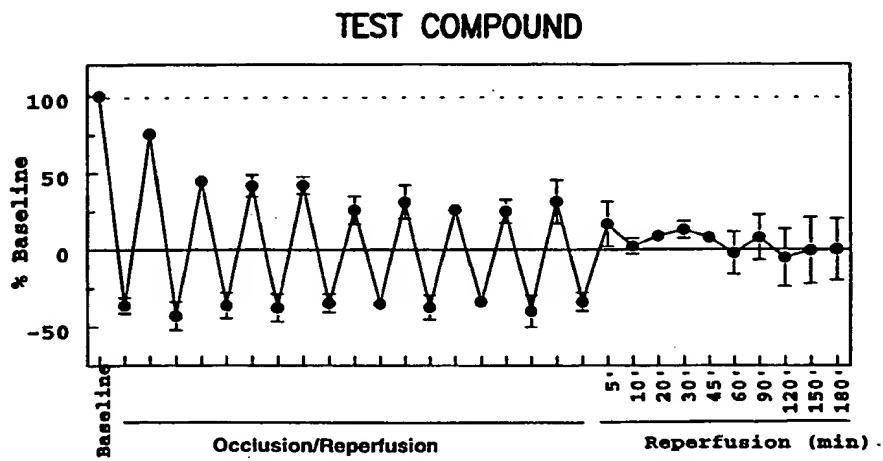
FIG. 20
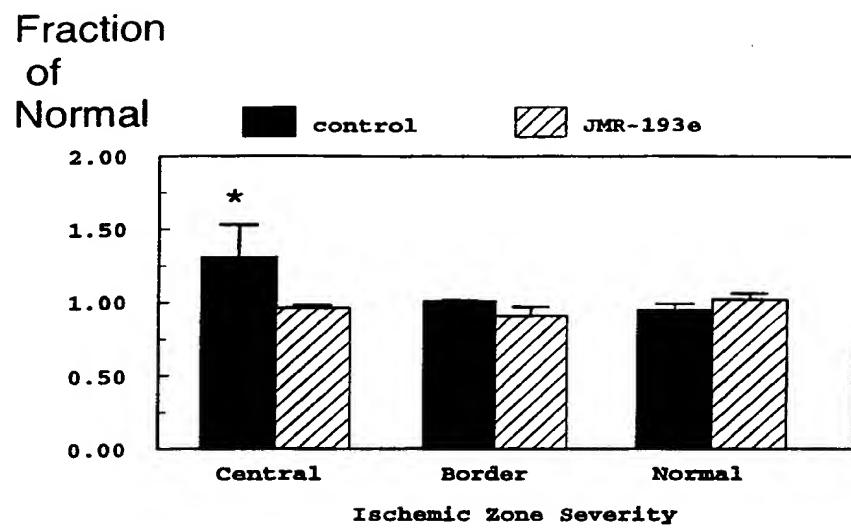
FIG. 21

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(51) International Patent Classification[7]: C07H 19/16, A61K 31/70, A61P 29/00

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09/333,387	15 June 1999 (15.06.1999)	US
60/151,412	30 August 1999 (30.08.1999)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/118,029 (CIP)
Filed on	1 February 1999 (01.02.1999)
US	60/124,316 (CIP)
Filed on	12 March 1999 (12.03.1999)
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Filed on	10 May 1999 (10.05.1999)
US	60/135,573 (CIP)
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(72) Inventors; and
(75) Inventors/Applicants (for US only): LINDEN, Joel, M. [US/US]; 207 Harvest Drive, Charlottesville, VA 22903 (US). SULLIVAN, Gail, W. [US/US]; 568 Taylor's Gap Road, Charlottesville, VA 22903 (US). SAREMBOCK, Ian, J. [US/US]; Box 158, Charlottesville, VA 22903 (US). MACDONALD, Timothy [US/US]; 2625 Jefferson Park Circle, Charlottesville, VA 22903 (US). OKUSA, Mark [US/US]; 2250 Cornwall Road, Charlottesville, VA 22901 (US). KRON, Irving, L. [US/US]; 155 Terrell Road East, Charlottesville, VA 22901 (US). SCHELD, W., Michael [US/US]; 2075 Earlysville Road, Earlysville, VA 22936 (US).

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(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent

(54) Title: COMPOSITIONS FOR TREATING INFLAMMATORY RESPONSE
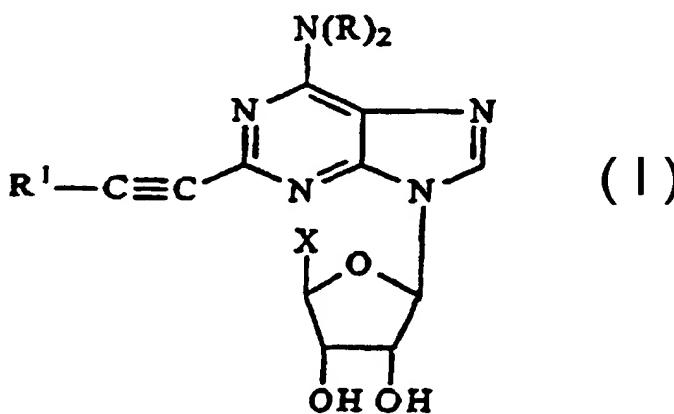
(57) Abstract: Compounds and methods are provided to treat inflammatory conditions with A 2a adenosine receptor antagonists of formula (I).

(I)

(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C07H19/16   A61K31/70   A61P29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C07H   A61K   A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 593 975 A (CRISTALLI GLORIA) 14 January 1997 (1997-01-14) cited in the application claim 1	1,17,49
A	BARALDI, PIER GIOVANNI ET AL: "Synthesis and Biological Activity of a New Series of N6-Arylcarbamoyl, 2-(Ar)alkynyl-N6-arylcarbamoyl, and N6-Carboxamido Derivatives of Adenosine-5'-N-ethyluronamide as A1 and A3 Adenosine Receptor Agonists" J. MED. CHEM. (1998), 41(17), 3174-3185 , XP002149470 scheme 4 page 3179, left-hand column, paragraph 2	1,17,49

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
6 October 2000	20/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Held, P

Form PCT/ISA/210 (second sheet) (July 1992)

page 1 of 2

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MAGER, PETER P.: "Neural network approaches applied to selective A2a adenosine receptor agonists" MED. CHEM. RES. (1998), 8(6), 277-290 , XP000952528 page 277 table 1 figure 1	1,17,49
P,X	OKUSA, MARK D. ET AL: "Selective A2A adenosine receptor activation reduces ischemia-reperfusion injury in rat kidney" AM. J. PHYSIOL. (1999), 277(3, PT. 2), F404-F412 , XP000952524 page F404	1,4,5,7, 8,10,11, 13, 17-22, 24,27, 28,30,49

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5593975	A	14-01-1997	IT	1254915 B	11-10-1995
			AT	193299 T	15-06-2000
			DE	69328722 D	29-06-2000
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